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VEGETATIVE PROPAGATION OF DERRIS AND LONCHOCARPUS WITH THE AID OF GROWTH SUBSTANCES

WILLIAM C. COOPER[1]

Introduction

The effectiveness of growth substances in inducing root formation on cuttings of the rotenone-bearing plants, *Derris elliptica* var. Sarawak Creeping and *Lonchocarpus utilis*, has been determined to provide information that may be of value in the culture of these crops in the tropics of Latin America. Both species are propagated exclusively by cuttings, so that any treatment which stimulates rooting may be important in their commercial production.

The main emphasis of the present investigation was placed on *Derris*, because of the urgency to develop some propagating procedure that would utilize the large quantities of small stems which have not proved satisfactory in field plantings and are largely discarded as useless. Considerable data, however, have also been obtained on *Lonchocarpus*.

The facilities of the Puerto Rico Experiment Station, U.S.D.A., at Mayaguez, P.R., were made available to the Office of Foreign Agricultural Relations for these investigations. The extensive collections of *Derris* from the Far East and of *Lonchocarpus* from the Western Hemisphere which have been assembled at this experiment station for observation and test made this location ideal for the present investigation. Furthermore, the work was done at a time when this station was actively engaged in distributing large quantities of planting stock of *Derris* to growers and experiment stations in Central and South America.

[1] Horticulturist, Office of Foreign Agricultural Relations, U.S. Department of Agriculture.

METHODS.—Four synthetic growth substances known to induce root formation on other species of plants were applied to the cuttings by the concentrated-solution dip technique (1). The compounds used were indoleacetic acid, indolebutyric acid, α-naphthaleneacetic acid, and α-naphthalene acetamide. Briefly, the method consists of dipping ½ inch of the base of the cutting for 5 seconds in a 50% ethyl-alcohol solution containing from 1 to 5 mg. per ml. of growth substance. The method was found to be equally as effective in the rooting of *Derris* cuttings as the other standard methods which have been used.

The propagating facilities consisted of muslin-shaded greenhouse benches, sash-covered greenhouse benches, and open-field nursery beds. The greenhouse benches, constructed of wood, were 30 feet long, 3 feet wide, and 1½ feet high. They contained approximately 10 inches of a fine yellow sand with a pH of 7.0. The open-field nursery beds were the same as those used by MOORE (4) at the Puerto Rico Station for the propagation of *Derris*. They consist of flat-topped ridges about 8 inches high and 3 feet wide, separated by drainage ditches about 1 foot wide. In these beds the small stem cuttings were planted about 1 inch apart in cross rows 6 inches apart. They were placed apical end uppermost at a 45° angle and almost completely covered with soil.

Experiments on Derris

Derris, a creeping leguminous plant, sends out numerous vines many feet in length. By the time the roots, which contain the rotenone, are ready for harvest (usually after $1\frac{1}{2}$–2 years), the vines on top of the ground have grown into a tangled mat. In the case of the Sarawak Creeping variety, which is the principal one planted at Mayaguez, most of the vines on the under side of the mat have shed their leaves and are more or less mature woody stems of varying size. The leafy vines on the top layer of the mat are mostly thin, semihard, greenwood stems.

This mat of leafy and leafless vines is cleared from the field just before harvesting the roots. In commercial practice no attempt has been made to use the greenwood leafy cuttings as propagating material. Only the mature leafless stems are salvaged, and, of these only the larger stems the diameter of a pencil ($\frac{1}{4}$ inch) have proved satisfactory planting material for open-field nursery beds. Usually only about 10–30% of the thin stems produce plants, while 90% or more of the larger stems are productive. Many of the thin-stemmed cuttings rotted in the beds. Others formed shoots at the top but few if any roots, and eventually died. It was thought that a growth-substance treatment might stimulate the rooting of these leafless small-stem cuttings and thereby produce a higher percentage of good plants than is obtained without treatment.

Leafless cuttings

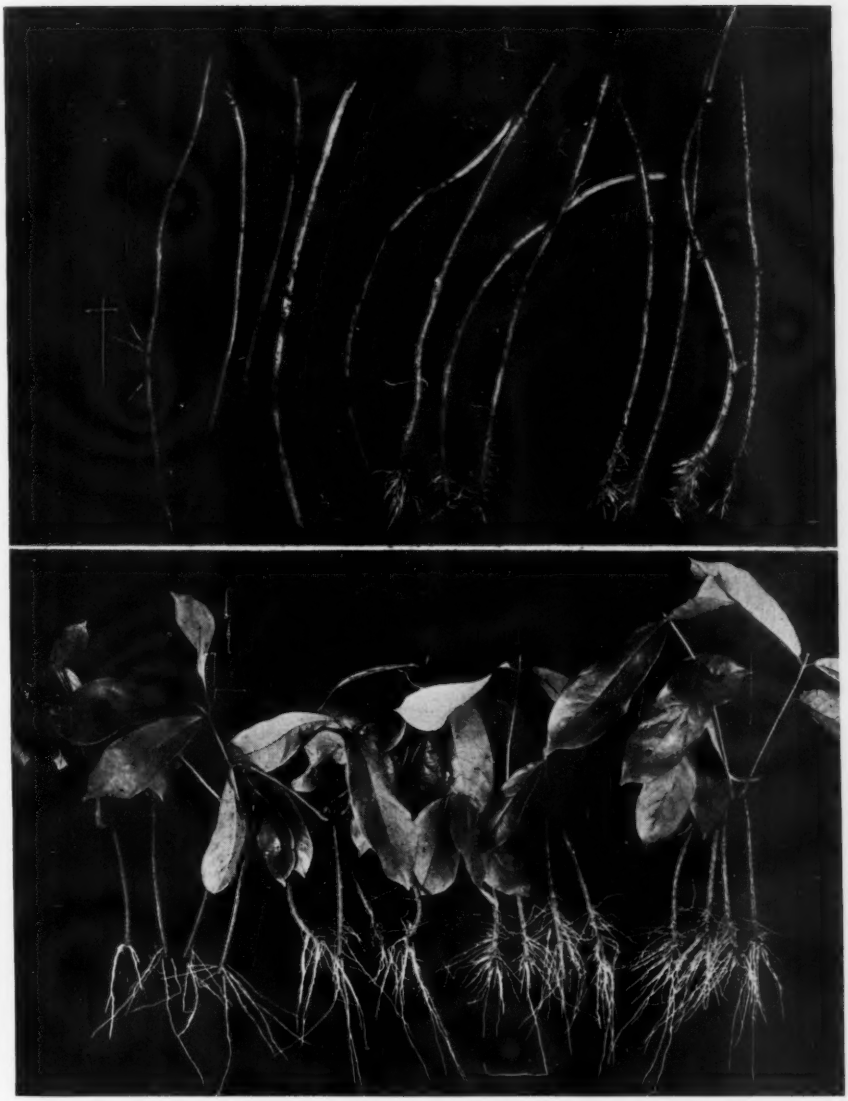
Effectiveness of growth substances on rooting.—The cuttings were prepared in the usual manner as practiced at the Station (4). This consists in cutting the vines into 3-foot lengths, arranging them into bundles of 300 each with basal ends together, tying the bundles securely at 6-inch intervals, and sawing them into bundles of cuttings 12 inches long.

A bundle of 300 cuttings was used for each treatment. Each growth substance was tested at 0, 1, and 2 mg. per ml. of solution. The cuttings were planted at once in field nursery beds, and twenty-five were dug up from each lot at 1-week intervals for examination.

At the end of 2 weeks the controls had formed an average of 2.4 roots per cutting on 33% of the cuttings, the remaining 67% having no roots. Those indoleacetic-acid treated showed no increase in rooting over the controls, while cuttings treated with indolebutyric acid, naphthaleneacetic acid, and naphthalene acetamide showed considerable increase in rooting. All three substances induced roots on approximately 75% of the cuttings when used at a concentration of 1 mg./ml. and on 88–100% when used at 2 mg./ml. The number of roots induced varied considerably but averaged about twenty for the 1 mg./ml. and thirty for the 2 mg./ml. solutions.

There appeared to be no very significant difference in the effectiveness of the three substances at these concentrations. Napthaleneacetic acid at the 2 mg./ml. concentration did cause slight injury to the base of the cuttings, however, while no injury was noticeable at this concentration for the other two substances. Typical cuttings treated with the indolebutyric-acid series are illustrated in figure 1.

The cuttings used in the preceding experiments were selected for uniformity and were classed as mature stems with yellow, or partially etiolated, bark. In any given bundle of field-run cuttings there may be other types of stems that respond differently to treatment. In par-



FIGS. 1, 2.—Fig. 1 (above), leafless cuttings of *Derris* 3 weeks after treatment with indolebutyric acid. Left to right: 50% alcohol (control); 1 mg./ml.; and 2 mg./ml. Planted in silty-clay loam. Fig. 2 (below), comparative growth of root systems on leafy and leafless cuttings 5 months after treatment with indolebutyric acid. Left to right: leafy 50% alcohol (control); leafy 2 mg./ml.; leafy 5 mg./ml.; leafless 50% alcohol (control); and leafless 2 mg./ml. Tops removed before photographing.

ticular, green stems were found to require higher concentrations to induce rooting than was required for the yellow-bark type of stem. The minimum effective concentration of indolebutyric acid for green stems was found to be 2 mg./ml.

AFTEREFFECTS OF GROWTH SUBSTANCES.—Data obtained from a detailed inspection of fifty or more of treated and control plants 3–4½ months old from twelve different locations on the experiment station are given in table 1. These data show that the average percentage of well-rooted plants with vigorous top growth was low, only 30%, and was the same for treated and controls. The actual number of cuttings that had died was greater for the treated (40%) than for the control lots (32%).

These results obtained in the field were checked under slightly more favorable conditions, whereby the cuttings were carefully graded, a smooth knife-cut at the base was substituted for the saw cut, and planting was in a silty, well-drained loam. Under these conditions both control and indolebutyric-acid (2 mg./ml.) treated cuttings grew better than in the regular field experiments, but the percentage of good plants after 3 months was less for the treated plants (49%) than for the controls (71%). As illustrated by the three cuttings on the right in figure 5, there was no sign of stem injury from the treatment. Most of the roots induced by the treatment simply stopped growing after they reached about ½–1 inch in length.

TABLE 1

EFFECT OF GROWTH SUBSTANCES ON SURVIVAL OF LEAFLESS SMALL-STEM CUTTINGS OF DERRIS PLANTED IN THE FIELD

Exp. no.	Growth substance	Concentration (mg./ml.)	Months after planting	No. of cuttings inspected	Percentage well-rooted with leafy top		Percentage dead	
					Control	Treated	Control	Treated
1.	Naphthaleneacetic acid	1	4	50	27	39	33	33
2.	Naphthaleneacetic acid	2	4	50	27	45	33	18
3.	Naphthaleneacetic acid	1	4.5	50	32	40	16	23
4.	Naphthaleneacetic acid	2	4.5	50	32	29	16	15
5.	Naphthaleneacetic acid	2	4.5	50	32	10	16	65
6.	Naphthalene acetamide	2	3	80	69	31	24	51
7.	Indolebutyric acid	1	4	79	24	20	35	39
8.	Naphthaleneacetic acid	1	4	82	24	32	35	33
9.	Naphthaleneacetic acid	2	4	91	24	32	35	43
10.	Naphthaleneacetic acid	2	4	56	24	5	35	55
11.	Naphthalene acetamide	1	4	67	24	33	35	48
12.	Naphthalene acetamide	2	4	85	24	12	35	49
	Average				30	29	32	40

LEAFY CUTTINGS

EFFECTIVENESS OF INDOLEBUTYRIC ACID ON ROOTING.—The leafy cuttings used were similar to the greenwood leafless cuttings, except that one leaf, trimmed to three leaflets, was left at the apex of each cutting. Only indolebutyric acid was used. After treatment, the cuttings were inserted in sand in the sash-covered greenhouse benches, since pre-

vious trials had shown that the leaves could be kept alive only in closed sash-covered frames.

Figure 2 shows that control leafy cuttings produced about five roots per cutting after a 3-week period in the frames. Treatments with 1, 2, and 5 mg./ml. concentrations of the acid were definitely effective in increasing the number of roots as compared with the controls. From root counts made on twenty-five cuttings of each treatment, the average number of roots per cutting for 1 mg./ml. was twenty-five; for 2 mg./ml. was thirty-four; and for 5 mg./ml. was sixty. No injury at the base of the cuttings was induced by any of these treatments.

The 2 mg./ml. solution was also used on leafless as well as on leafy cuttings, and this solution induced only about half as many roots on leafless stems as on leafy ones (fig. 3). Both types of cuttings were grown in the sash-covered frame.

AFTEREFFECTS OF GROWTH SUBSTANCES.—Rooted leafy cuttings grew well when transplanted directly from the propagating frames to the field nurseries. Figure 4 shows the controls and the treated lot (5 mg./ml.) 1 month after transplanting. All roots on both lots were growing vigorously. There was no sign of root degeneration.

Figure 5 shows a comparison of root growth on treated and control leafy and leafless cuttings 5 months after planting. The roots on the leafy cuttings were still growing vigorously. These plants were from the same lot of cuttings as illustrated in figures 2 and 4. The root system on the treated cuttings was much more extensive than on the controls.

In comparing the treated leafy and leafless cuttings (top growth removed from all plants to facilitate photographing), it is seen that while root growth was vigorous and extensive on treated leafy cuttings, it was very weak on treated leafless ones. Most of the roots induced by the treatment on leafless cuttings had either died or were in about the same state of growth as they were 4 months earlier. Thus it appears that vigorous root growth on treated cuttings was associated with the presence of leaves.

Another beneficial after-effect resulting from the treatment of leafy cuttings is the greater survival of the plants, as compared with controls, on transplanting. In a large-scale experiment, 2000 untreated leafy cuttings and 2000 treated (5 mg./ml.) cuttings were transplanted to field nursery beds after a 3-week period in the propagating frames. The transplanting took place during a dry hot spell in late August, and it was necessary to apply partial shade for 3 days. Counts made on leaf-drop 10 days after transplanting showed that 95% of the treated plants held their leaves and were growing well as compared with 85% for the untreated cuttings. The fifty-five more roots per cutting on the treated plants (five roots on the controls) appeared to be a definite aid in quick establishment in the new environment.

Experiments on Lonchocarpus

Lonchocarpus, like *Derris*, is a member of the legume family. It has an erect growth habit, becoming almost treelike after 2 years (fig. 6). The plant, native to South America, furnished about 50% of the United States supply of rotenone before the war and is today our principal source of this material. The two high rotenone-yielding clones of *Lonchocarpus utilis* growing at the Puerto Rico Station, and with which this investigation was concerned, are reported (3) to have rotenone contents of 14.02 and 16.5%.

The plant is propagated by stem cut-

tings made by sawing the long woody stems, usually 1 inch or more in diameter, into cuttings that have several nodes and are 12–18 inches in length (5). In this investigation, however, owing to shortage of propagating material, the stems were sawed into 6-inch lengths.

In South America it is the usual practice to set the cuttings directly in the field, where mortality up to 50% is not unusual (5). In Puerto Rico the mortality of field-planted cuttings of the high-yielding clones was much greater than 50%, and often none of them survived.

FIGS. 3, 4.—Fig. 3 (above), leafy cuttings of *Derris* 3 weeks after treatment with indolebutyric acid. Left to right: 50% alcohol (control); 1 mg./ml.; 2 mg./ml.; and 5 mg./ml. Fig. 4 (below), comparative rooting of leafy and leafless cuttings of *Derris* 3 weeks after treatment with 2 mg./ml. indolebutyric acid. Left to right: leafless control, leafless treated, leafy control, leafy treated.

Accordingly, the greenhouse benches were used exclusively in this investigation.

Leafless stem cuttings

The four growth substances used in the experiments with *Derris* were tested on leafless cuttings of *Lonchocarpus* at 0, 1, 2, and 5 mg./ml. concentrations. The control cuttings developed an average of about five roots each. The indoleacetic acid at all concentrations failed to increase the number of roots over that in the controls. On the other hand, the three other compounds were especially effective in stimulating rooting. The 1 mg./ml. solutions showed a slight increase in the number of roots, while the 5 mg./ml. solution of all three substances induced as many as fifty husky roots per cutting without causing noticeable injury to the bases. The results were complicated, however, in that a great many of the cuttings succumbed from a decay (undetermined species of *Penicillum*) occurring at the top of the cuttings. The first evidence of this mold at the apex occurred within 6 days after placing the cuttings in the propagator. It was often preceded by a resinous exudate from the trachea tubes at the upper cut surface. The bark around the top of the cuttings was usually killed within 2 or 3 weeks.

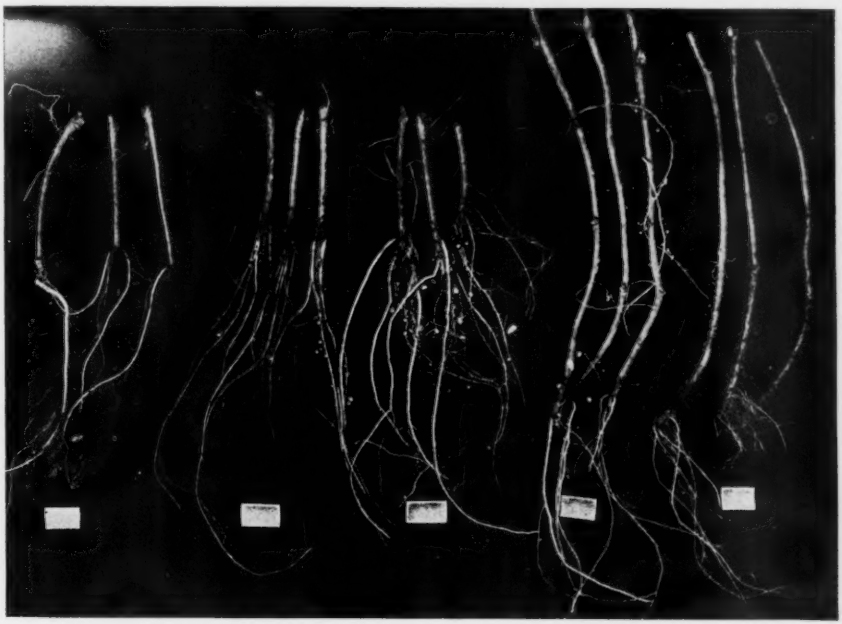


Fig. 5.—Leafy cuttings of *Derris* 1 month after transplanting to nursery beds. Plants on left: 5 mg./ml. indolebutyric acid; plants on right: 50% alcohol (control).

In general, the decay at the top occurred more frequently on treated cuttings than on controls. It was definitely greater on cuttings treated with a 5 mg./ml. solution of indolebutyric acid (table 2; fig. 7). At the same time there was pronounced bud inhibition in the treated cuttings. It may be that the decay is in some way connected with the

bud inhibition induced by the treatment.

Cutting material was so limited that it was not possible in this investigation to determine whether a growth-substance treatment could be developed that was effective in increasing root formation without inducing excessive bud inhibition and top decay. Until this problem is further investigated it seems inadvisable to use the growth substances on this type of cutting. Untreated material, under the conditions of these experiments, usually developed one or more (average five) roots on approximately 70% of the cuttings after 6–8 weeks and at the same time produced vigorous top growth.

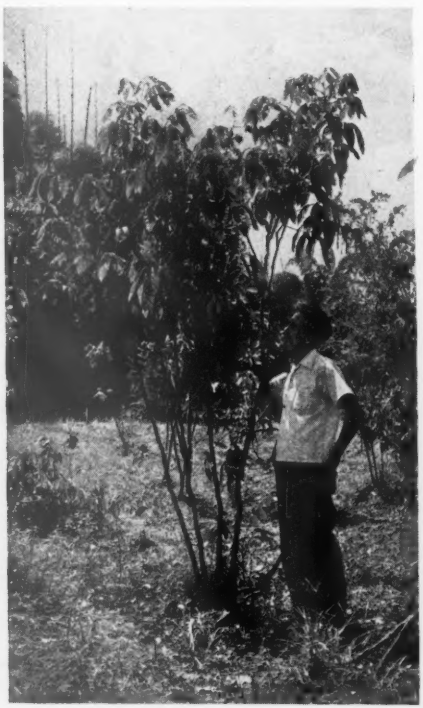


FIG. 6.—Typical plant (3 years old) of one of the high rotenone-yielding clones of *Lonchocarpus* now growing at Puerto Rico Experiment Station.

LEAFY VINE CUTTINGS

Plants of *Lonchocarpus* after 2–3 years of upright growth will frequently produce extensive vinelike growth at the top of the plant. Such vines can be cut into a number of pieces containing one

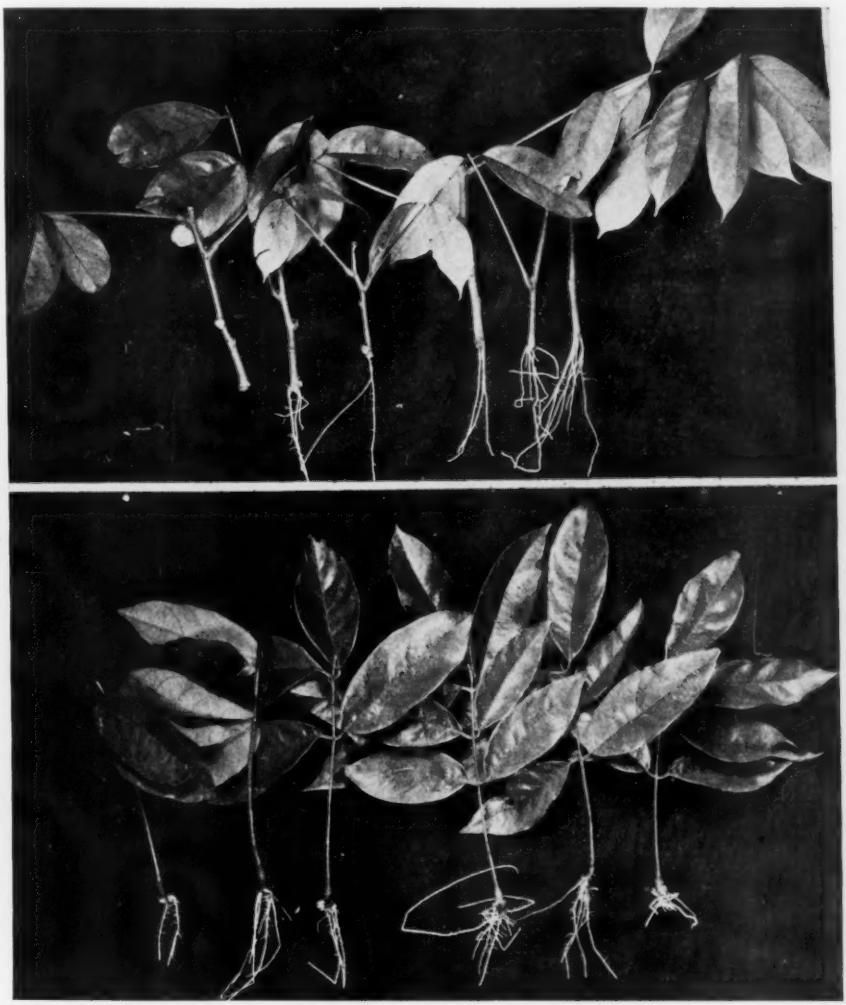
TABLE 2

NEW SHOOT GROWTH, NUMBER OF ROOTS, AND INCIDENCE OF DECAY IN NAPHTHALENE-ACETAMIDE TREATED AND CONTROL CUTTINGS OF LONCHOCARPUS

Concentration (mg./ml.)	No. of weeks buds dormant	Ave. length of new shoots after 7 weeks (cm.)	Ave. no. of roots per cutting after 7 weeks	Percentage cuttings showing top decay
0.....	2	68.0	4.9	10
5.....	6	0.5	50.0	60

FIG. 7.—Leafless cuttings of *Lonchocarpus* 6 weeks after treatment with α-naphthalene acetamide. Left to right: 5 mg./ml.; 50% alcohol (control); 2 mg./ml.

or more nodes with a leaf at the apex and be used as cuttings. Rooting obtained on treated and untreated leafy vine cuttings after 3 weeks in sash-covered frames is shown in figure 8. A treatment consisting of 2 mg./ml. indolebutyric acid induced an average of five roots per cutting as compared with two for untreated cuttings.



FIGS. 8, 9.—Fig. 8 (above), leafy vine cutting of *Lonchocarpus* 3 weeks after treatment. Left to right: 50% alcohol (control); 2 mg./ml. of indolebutyric. Fig. 9 (below), leaf-bud cuttings of *Lonchocarpus* 3 weeks after treatment. Left to right: 50% alcohol (control); 2 mg./ml. indolebutyric.

It has not yet been determined into what type of plant these leafy vine cuttings will develop, and an opinion as to

their value as propagating material is reserved until further investigation.

Leaf-bud cuttings

Another method of vegetative propagation now being used extensively, particularly in ornamental plants, is leaf-bud cuttings (6). The technique consists of taking a bud with a single leaf attached, just as though it were to be used in shield budding. The bud is inserted in sand so that the shield of wood is just covered and the leaf is exposed above the sand. In these experiments the leaf-bud cuttings were placed in the sash-covered frames.

Results obtained with treated and untreated leaf-bud cuttings are illustrated in figure 9. The leaf buds were taken from the leafy shoots at the top of the plants and not from leafy vines. The cuttings were treated by dipping the bud and the lower part of the petiole in the growth-substance solution, which in this experiment consisted of indolebutyric acid, 2 mg./ml. In 4 weeks the leaf buds produced about five long heavy roots, and there was no great difference in the number produced on treated and control cuttings. The buds on these cuttings had not started to grow after 1 month but were still alive and healthy. If these buds develop satisfactorily, this method has a definite application where there is scarcity of propagating material. Each node of the leafy top growth of the plant (fig. 6) may be used as a leaf-bud cutting.

Discussion

Under the conditions employed in these experiments, the hormone treatment of leafless cuttings of *Derris* and *Lonchocarpus* appears to be more detrimental than beneficial. All three substances were effective in inducing roots, but the cuttings failed to develop properly.

In case of *Derris*, most of the roots simply failed to grow beyond an inch or so in length. Often the roots would wither and die. Always accompanying this rooting response was pronounced bud inhibition. In *Lonchocarpus* there was the same abundance of roots induced and the same pronounced bud inhibition. But there was an additional complicating factor resulting from invasion of the cuttings at the apex by a *Penicillum* mold that usually killed the cuttings within 6 weeks.

This collapse of leafless cuttings of *Derris* and *Lonchocarpus* as a result of treatment was induced without any evidence of external injury to the tissues of the base of the cutting. Such killing of the base can be induced by the use of more concentrated solutions. The disturbance concerned with in this discussion is apparently an internal physiological one created by the treatment and apparently associated with excessive numbers of roots.

It has been demonstrated that growth-substance treatment apparently causes mobilization of food reserves at the base of cuttings, where they are utilized in root formation (2). In treated leafless cuttings, where fifty or more roots are frequently initiated on a single cutting, there is bound to be a heavy demand for the stored food materials. With leafy cuttings this demand can be taken care of by continued photosynthesis in the leaves. In leafless cuttings there is no way to obtain additional food supply except by pushing out new shoots. In itself, however, the treatment induces temporary bud inhibition. Thus the collapse of treated leafless cuttings of *Derris* may be due, at least partly, to the starva-

tion caused by the excessive number of roots depleting the reserve food supply.

Decay of the treated cuttings of *Lonchocarpus* was hastened by the entrance of the *Penicillum* at their apex, indicating that somehow the treatment made the cuttings more susceptible to attack; but whether treatment directly stimulated growth of the decay organism is not known.

Recently STUART and MCCLELLAN (7) reported that the severity of narcissus basal rot was increased by the use of growth substances and that the substances stimulated the growth of pure cultures of *Fusarium oxysporum*, the organism causing basal rot. They inferred that the basal rot of the treated bulbs was due to the stimulating effect of the substances on growth of the organism.

From this conclusion it might be suspected that the growth substances stimulated the *Penicillum* decay in *Lonchocarpus*. At the same time the explanation for the increased incidence of decay might lie in the increased susceptibility of the apex of the cutting due to depleted food reserves in that section. Once the buds began to grow there was usually no more decay.

Use of more dilute solutions on leafless cuttings should be considered. Perhaps the ideal growth-substance effect on leafless cuttings might be to produce only a slight increase in number of roots over controls and not to induce sufficient roots to cause ill after-effects. In general, however, the range in concentration between no increase in rooting and excessive rooting is rather small with leafless *Derris* and *Lonchocarpus*. Also, in any given bundle of field-run cuttings there may be several types that respond differently to the treatment. At the present stage of investigation it would not be advisable to use a treatment stronger than 0.5 mg./ml. for leafless *Derris*. With leafless *Lonchocarpus* perhaps no treatment should be used until the problem has been further investigated.

Summary

1. Cuttings of *Derris* and *Lonchocarpus* were treated with indoleacetic acid, indolebutyric acid, α-naphthaleneacetic acid, and α-naphthalene acetamide by the concentrated-solution dip technique.

2. Indoleacetic acid did not stimulate rooting at concentrations of 1 and 2 mg./ml., while the other three compounds were highly effective at these concentrations on both *Derris* and *Lonchocarpus*.

3. Roots produced on treated leafless cuttings of *Derris* did not grow well, while those induced on treated leafy cuttings grew exceptionally well.

4. The incidence of decay at the top of leafless cuttings of *Lonchocarpus* was greater in treated than in untreated cuttings.

5. Growth substances stimulated the rooting of leafy cuttings made from the vinelike growth at the tops of *Lonchocarpus*.

6. Treated and untreated leaf-bud cuttings of *Lonchocarpus* rooted well.

The writer is indebted to Dr. KENNETH A. BARTLETT and Dr. RUFUS H. MOORE of the Puerto Rico Experiment Station, U.S.D.A., Mayaguez, P.R., for their generous co-operation during the course of these experiments.

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VARYING STRUCTURE OF CONIFER LEAVES IN DIFFERENT HABITATS

ERNEST L. STOVER

Introduction

Studies of variations in the structure of broad-bladed leaves of plants in different habitats have been made by CLEMENTS (4), STARR (16), HANSON (6), HAYDEN (8), MCDOUGALL (12), and others. These investigations have shown that the leaves of zeromorphic forms of nonsucculent mesophytes are thicker, have more rows of palisade cells, more sclerenchyma, a thicker cuticle, and more mucilaginous and resinous substances in the mesophyll cells than in the mesomorphic forms. The mesomorphic leaves have larger air spaces than the xeromorphic leaves. The xeromorphic broad leaves develop under conditions of bright light, dry air, and low water supply—or some combination of these factors.

A number of investigators have shown that leaves with many palisade cells and small air spaces (xeromorphic forms) transpire more water per unit of epidermal surface than leaves with larger air spaces (1, 2, 3, 7, 11, 14, 17).

TURRELL (17) has measured the internal leaf surface of various plants and found that the more compact leaves with numerous small air spaces and usually more layers of palisade cells have greater internal evaporating surface than leaves with larger air spaces. Other things being equal, this greater internal surface of xeromorphic leaves can account for their greater transpiring power.

Studies have been made of the differences in the structure of coniferous leaves of nursery stock by KORSTIAN (9) and LARSEN (10). It was found that conifer leaves grown in shade are thinner, have a thinner epidermis and cuticle, more sponge tissue, and the guard cells are nearer the surface of the leaf than in those grown in brighter light.

The writer has been interested in the differences in the structure of conifer leaves as affected by both age and habitat. The summer of 1940 was spent in the University of Wyoming Science Camp in the Medicine Bow Mountains, and there studies were made of the leaves of *Abies lasiocarpa* (Hook.) Nutt., *Picea engelmanni* (Parry) Engelm., and *Pinus contorta* Loud. The leaves of *A. lasio-*

carpa and *P. engelmanni* were collected from both the lee and windward sides of the Krummholz at about 11,000 feet, from a mesophytic protected area between 9000 and 10,000 feet, and from the dry granite outcroppings east of Laramie known as the Veedavoo Rocks. The specimens of *P. contorta* were collected from the most mesic and from the most xeric habitats found in the area. The xeric habitat was in the Veedavoo Rocks in the Laramie Range and the mesic habitat was in the Medicine Bow Mountains between 9000 and 10,000 feet.

In choosing specimens, care was taken to select average-sized leaves from a given tree. Comparisons are of leaves produced during the same year, since the size of needles varies a great deal from year to year.

In most textbooks the pine needle is the only conifer leaf figured, and usually only the cross-section is shown. So far as the writer knows, the last edition of the STRASBURGER text was the first to figure a portion of the longitudinal section of a pine needle. Longitudinal sections show that the "infolded cells"[1] are palisade-like. Cross-sections of a 2- or 3-year-old pine needle show the largest air spaces (stomatal cavities) adjoining the stomatal openings. But the air spaces in pine needles are much more extensive, for the longitudinal sections from both the xeric and mesic habitats show that the infolded cells of both pine and spruce are transverse plates of cells with varying sizes of air spaces between them.

The mesophyll of the alpine fir is totally without the infolded cells. These leaves show palisade-like cells in transverse as well as in longitudinal sections. This fir grows in relatively mesic habitats, and transverse as well as longitudinal leaf sections show numerous and relatively large air spaces.

The specimens of the three species investigated did not have more than two resin ducts. Those of *Picea engelmanni* are only in the basal half of the needles. The epidermal cells are thick-walled in all specimens except those of the current growing season. These young leaves show thickened cuticle, epidermal cell walls, and thickening of the hypodermal cell walls by the time the leaves reach their maximum size. The greatest thickening of cell walls occurs at the tip and at the base of the leaves, and the greatest number of cells of hypodermal sclerenchyma is at the base of the needles. Thickness of the cuticle varies with age and habitat. The thick-walled hypodermal cells vary in number with the species, the age of the needle, and the habitat. The smallest number occurs in the younger needles and in those growing in the mesic habitats. It is not known how new hypodermal cells originate; that is, whether they are from the division of other hypodermal cells or from the division of epidermal cells.

In the leaves of *Pinus* and *Picea* studied the endodermis is a continuous sheath of cells, and no "passage" cells were found. The endodermis in *Abies* is not a complete sheath of like cells, even in the bases of the leaves (fig. 11). As reported previously by SOAR (**15**), no Casparian strips were seen in either *Abies* or *Picea*. SOAR also reported that the radial walls of the endodermis were lignified and covered with a layer of suberin, and that the outer tangential walls are thicker than the inner in the specimens examined. SOAR found that the outer tangential walls of the endo-

[1] Species of *Pinus*, *Picea*, and *Larix* are the conifers in North America that have the "infolded" mesophyll cells. There are only a few real "infoldings" in *Picea engelmanni*.

dermis in most leaves of *Pinus* and *Picea excelsa* were lignified and the inner walls sometimes lignified, but that the inner walls may be of pure cellulose. The writer did not find that lignification occurred as SOAR has reported, for in these specimens the inner tangential walls and the radial walls are the first to be lignified, and the degree of lignification increases with the age of the leaves in both mesic and xeric habitats.

The pericycle (transfusion tissue) is made up of three types of cells: cells with bordered pits like those of the tracheids; very thick-walled fiber cells more abundant in xeric habitats; and larger parenchyma cells that retain their cell contents and are often filled with an amorphous substance. All these cells become completely lignified in the oldest and more xeromorphic leaves.

The xylem and phloem in these species of *Pinus* and *Abies* are in two areas of the stele and in *Picea* are in one area, although the phloem is separated into two areas by a prominent phloem ray—except at the base of the leaf.

Methods

Measurements of the leaves were made with Vernier calipers. Living leaves direct from the trees were measured before any shrinkage took place as the result of transpiration. Free-hand sections, both cross and longitudinal, were examined on the day the leaves were collected. Material for further study and for making the stained microscopic slides was preserved in alcohol and formaldehyde with Tergitol. Three cubic centimeters of formaldehyde (40%) was added to each 100 cc. of 30% alcohol. One drop of Tergitol penetrant 7 (Carbide and Carbon Chemicals Corporation) was added to each 100 cc. of the solution. The leaves were held in this for several months.

The leaves were dehydrated by using cellosolve, two changes of 2–3 hours each, and infiltrated with paraffin by means of toluene to which was added a small amount of chloroform (about 1 cc.). The amount of chloroform can be increased to speed up infiltration. The paraffin has a melting point of 56° C. The imbedding mixture, locally called rubber-paraffin, is the 56° paraffin to which was added a mixture of paraffin, raw rubber, and bayberry wax. The imbedding formula is modified from that of HANCE (5). Twenty grams of raw rubber is dissolved in 100 gm. of paraffin and to this is added 2 gm. of bayberry wax. The imbedding paraffin is made by adding 5–10 gm. of the rubber-bayberry mixture to each 100 cc. of melted 56° C. paraffin.

The sections were fixed to the slides with the Szorndathy fixative. Some slides were stained with safranin and fast green, and others with the Haidenhain haematoxylin iron-alum method. Dehydration during staining was by cellosolve, with clearing in wintergreen oil and xylene.

Investigation

ABIES LASIOCARPA (HOOK.) NUTT.

Table 1 shows that the size of leaves of this species varies as do broad leaves, but within smaller limits. The needles in the most mesic habitat are the longest and thinnest and those in the most xeric habitat the shortest and thickest. The most unexpected difference occurs in the needles of the trees of the Krummholz. There the needles of the windward (west) side of these wind-blown trees have more nearly the size of the leaves from the mesic habitat than have those of the lee

(east) side. This unexpected mesomorphic condition is shown also in the internal structure (table 2).

The abscission of leaves was found to vary with the habitat. The leaves remain on the trees for the greatest number of years in the most mesic habitat, up to 17 years. They remain on the trees for the shortest time in the most xeric habitat. These live for as long as 10 years but many fall before that. The oldest needles found on the windward side of the Krummholz were 12 years old, while those on the lee side live as long as 16 years. This seems to contradict the facts of size and internal structure, which indicate that the leaves on the windward side of a Krummholz are more like those from a mesic habitat than those in the lee side. Abscission is apparently controlled by factors not directly concerned with those causing variations in size and internal structure of evergreen needle leaves.

The difference in thickness of the leaf is more evident to the eyes in the xeric habitats than the measurements would indicate. Leaves in the mesic habitats are always much flattened and often have the appearance of a midrib and lamina, while in xeric habitats the lower surface is more nearly a half cylinder.

Table 2 gives the approximate measurements of the internal structures of the alpine fir needles from the same habitats as listed in table 1. The thickness of the cuticle is greatest in the most xeric habitat and tends to be thicker on the lee side of the Krummholz than on the windward side. The stomatal pits are shallowest in the mesic habitats, deepest in the most xeric, and have the same average depth on both sides of the Krummholz. Wall thickness of the epidermal and hypodermal cells is most pronounced in the leaves from the xeric habitats. The subsidiary cells of the guard cells show this variation to a marked degree. The outer epidermal cell walls are thicker than the inner in all leaves (figs. 1–3).

The greatest number of palisade-like cells and the smallest air spaces are in the leaves from the xeric habitats, as shown in the longitudinal sections (figs. 4–6). There is a difference in the size of the resin ducts, not only in different leaves but also in different habitats. The ducts of the smallest diameter are in the leaves from the most mesic habitats (fig. 1).

The veins are largest in the basal halves of the leaves; the measurements in table 2 are averages giving the approximate size of the largest cross-sections from each habitat. The largest veins are in the leaves from the xeric habitat. They are smaller in the most

TABLE 1

ABIES LASIOCARPA, SIZE OF LEAVES

Habitat	One year old	Two years old	Three years old	Four years old	Av. size for four years
	Length × Width × Thickness (mm.)	Length × Width × Thickness (mm.)	Length × Width × Thickness (mm.)	Length × Width × Thickness (mm.)	Length × Width × Thickness (mm.)
Alpine: Krummholz, exposed or windward side, 11,000 ft.	20.3 × 1.55 × 0.76	21.8 × 1.52 × 0.73	22.8 × 1.47 × 0.72	22.7 × 1.47 × 0.77	22 × 1.5 × 0.77
Alpine: protected or lee side	19.5 × 1.45 × 0.82	18.5 × 1.45 × 0.75	20.7 × 1.6 × 0.85	21.5 × 1.57 × 0.82	20 × 1.5 × 0.81
Mesic: near stream, 9000–10,000 ft.	27 × 1.5 × 0.65	26.1 × 1.5 × 0.7	23.3 × 1.5 × 0.65	25 × 1.4 × 0.65	27 × 1.5 × 0.66
Xeric: dry rocks, Laramie Range, 6000–7000 ft.	17 × 1.5 × 0.83	17.1 × 1.5 × 0.82	19.5 × 1.4 × 0.76	18.7 × 1.4 × 0.8	18 × 1.4 × 0.8+

TABLE 2

ABIES LASIOCARPA, INTERNAL STRUCTURE OF LEAVES

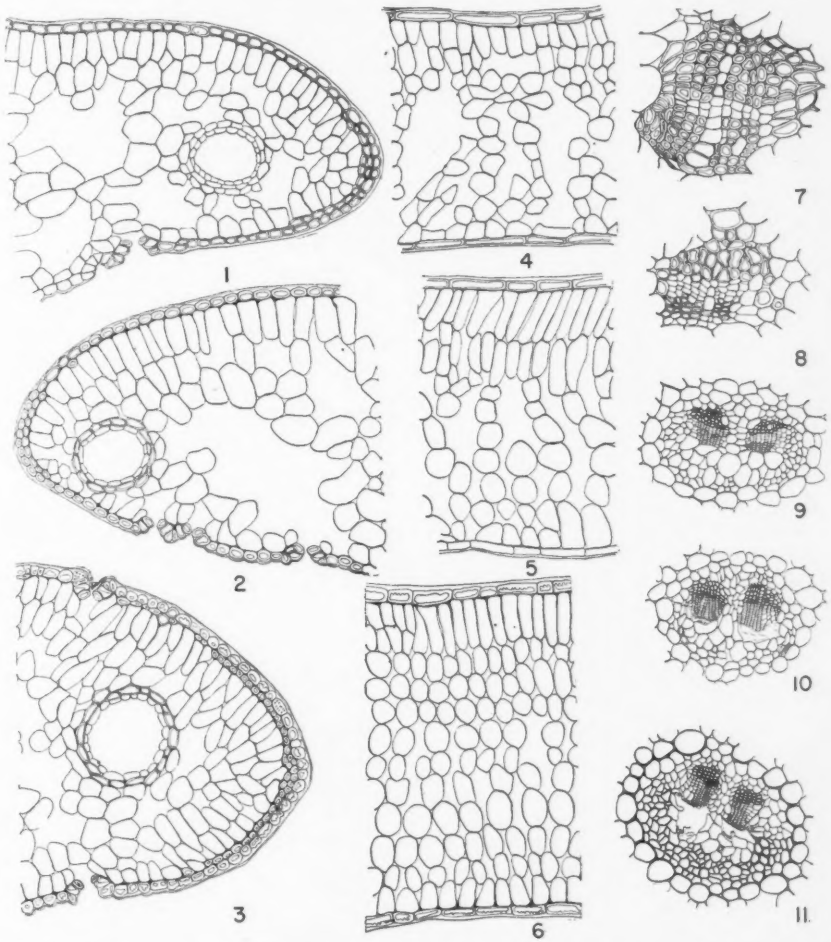
HABITAT	AV. THICKNESS OF CUTICLE (μ)	AV. DEPTH OF STOMATAL PIT (μ)	AV. THICKNESS OF HYPODERMAL CELL WALLS (μ)	GREEN MESOPHYLL, PALISADE-LIKE CELLS IN CROSS-SECTION	MESOPHYLL IN LONGISECTION	AV. DIAMETER OF RESIN DUCTS (μ)	SIZE OF VEIN IN CROSS-SECTION	AV. NUMBER OF XYLEM CELLS	AV. NUMBER OF PHLOEM CELLS	REMARKS
							Thickness × Width (μ)	Thickness × Width	Thickness × Width	
Alpine: Krummholz, windward side, 11,000 ft.	3.6–4.8	24	2.4–3.6	3+ layers, upper and lower	Larger air spaces than on lee side	40	270×360	4×8(1)‡ 6×12(2) 6×12(3)	6–7×8–9(1) 9–10×12–15(2) (outer crushed cells cannot be counted) (3)	Phloem cells with thickened walls
Alpine: Krummholz, lee side, 11,000 ft.	3.6–7.6	24	1.2–3.6	2–3 layers, upper and lower	More compact than on windward side	30	250×420	6–7×12–13(1) 5×11–12(2) 6–7×11–12(3)	9–10×11–12(1) 6–7×13–15(2) 9–10×13–15(3) (some crushed and destroyed)	Phloem cells with thickened walls
Mesic: 9000–10,000 ft.*	2.4–3.6	20	2.4	1 layer, upper	Largest air spaces	20	660×860	2–3×6–8(1) 5×8(2) 5–6×9–10(3)	6–7×9–12(1) 7–9×14–15(2) 9–13×12–13(3)	In some cases, middle lamella dissolves and older phloem goes to pieces or is crushed
Xeric: Laramie Range, 7000–8000 ft.†	4.8–7.2	36	3.6–4.8	3–4 layers, upper and lower	Air spaces but noticeably smaller than above	30	800×800	5×9–10(1) 5×9–10(4)	8–10×11–13(1) ?×9–11(4) (crushed cells cannot be counted)	In some cases, middle lamella dissolves and older phloem goes to pieces or is crushed

* Young leaves and leaves from mesic habitat lignified in xylem, slightly in pericycle and inner tangential walls of endodermis.

† Leaves from xeric habitat lignified in hypodermal cell walls, all of pericycle, xylem, ray between bundles, and inner tangential walls of endodermis.

‡ Numbers in parentheses indicate age in years.

mesic habitat and smallest in the stunted trees of the Krummholz. Counts of the cells of the xylem and phloem show some increase in the number of cells in both tissues after the first year. The greatest increase in number of conducting cells is in the leaves from the mesic habitats. In all leaves examined there were more phloem than xylem cells in the cross-sections, except in those in which the oldest phloem was crushed and apparently dissolved. The phloem cells from younger leaves and leaves of mesic habitats have the thickest walls. The cam-



FIGS. 1–11.—Needles of *Abies lasiocarpa:* Fig. 1, cross-section, 1 year, mesic habitat. Fig. 2, cross-section, 4 years, mesic. Fig. 3, cross-section, 4 years, xeric. Fig. 4, longisection, 1 year, mesic. Fig. 5, longisection, 4 years, mesic. Fig. 6, longisection, 4 years, xeric. Fig 7, cross-section of vein, 3 years, lee side of Krummholz. Fig. 8, cross-section of vein, 1 year, mesic. Fig. 9, cross-section of stele, 1 year, mesic. Fig. 10, same, 4 years, mesic. Fig. 11, same, 4 years, xeric.

bium is evident, even in the oldest needles, for it remains as an area of thinner-walled cells between the xylem and phloem, at least during July and August, when these leaves were collected. Figures 10 and 11 show cross-sections of veins from needles from mesic and xeric habitats. The confirming evidence that there is cambium growth is the actual increase in the number of cells of the xylem and phloem, rather than the appearance of the cells.

The oldest needles found on the trees in the xeric habitat were 12 years old, while those in the mesic habitats remained on the trees as long as 17 years. The oldest needles on the lee side of the Krummholz were 12–16 years old, while the oldest on the windward side were 9–12 years. The cuticle is thinner on the leaves of the windward side, the hypodermal cell walls tend to be less thickened, the air spaces as seen in the longitudinal sections are in general larger

TABLE 3

PICEA ENGELMANNI, SIZE OF LEAVES

HABITAT	ONE YEAR OLD	TWO YEARS OLD	THREE YEARS OLD	FOUR YEARS OLD	AV. SIZE FOR FOUR YEARS
	Length×Width× Thickness (mm.)	Length×Width× Thickness (mm.)	Length×Width× Thickness (mm.)	Length×Width× Thickness (mm.)	Length×Width× Thickness (mm.)
Alpine: Krummholz, windward side, 11,000 ft.	17 ×1.3 ×1.2	19 ×1.3 ×1.1	17 ×1.2 ×1.0	17 ×1.3×1.0	17.5×1.3×1.1
Alpine: Krummholz, lee side, 11,000 ft.	20 ×1.3 ×1.1	24 ×1.3 ×1.15	23 ×1.2 ×1.1	20 ×1.4×1.2	21.3×1.3×1.1
Mesic: near stream, 9000–10,000 ft.*	19.8×1.5 ×1.0	21.8×1.25×0.98	21.7×1.26×0.86	18.7×1.0×0.95	20.5×1.2×0.95
Xeric: high on Laramie Range (Veedavoo Rocks)	15.4×1.25×1.26	15.4×1.3 ×1.34	16.0×1.34×1.2	14.0×1.3×1.1	15.2×1.3×1.2

* Largest needles (23×1.7×1.3) found in mesic habitat.

PICEA ENGELMANNI (PARRY) ENGELM.

The needles of spruce in xeric and mesic habitats vary less than the fir needles. They are never flattened but always four-angled in transverse section. The leaves growing in the xeric habitats are shorter, generally thicker, and much more rigid than those in the mesic situation (table 3). The needles of the spruce of the Krummholz do not have the same relative sizes as do the fir needles. The spruce needles are longer and somewhat thicker on the lee side and shorter and somewhat thinner on the windward side. There is a tendency, even in the spruce, for the needles on the windward side to be somewhat more mesomorphic in internal structure than the needles on the protected or lee side (table 4).

than those in the leaves from the lee side, and the veins are smaller in cross-section. These comparisons indicate that these leaves are more nearly like those of mesic habitats. There is more lignification in the leaves on the windward side.

In the habitats below the alpine Krummholz, the greatest thickness of cuticle is in the drier habitats. There is less variation in the depth of the stomatal pits than in the alpine fir. Many of the stomatal pits in the leaves from the xeric habitats are filled with an amorphous material (fig. 14). These deposits are loosened in the preparation of microscopic slides and were found to be directly connected with the outermost layer of the cuticle. The epidermal and hypodermal cells are noticeably thicker-walled in the needles from the xeric habi-

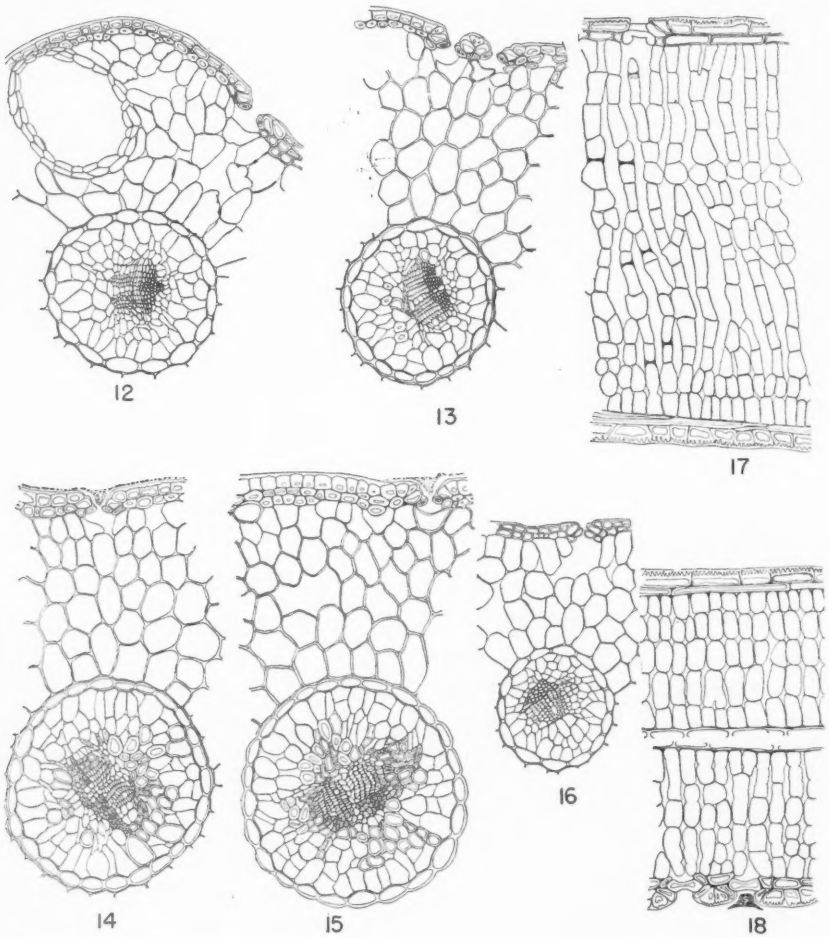
TABLE 4

PICEA ENGELMANNI, INTERNAL STRUCTURE OF LEAVES

Habitat	Av. thickness of cuticle (μ)	Av. depth of stomatal pit (μ)	Av. thickness of hypodermal cell walls (μ)	Green mesophyll, palisade-like cells in longisection	Approx. size (average) of cross-section of vein (μ)	Av. number of xylem cells	Av. number of phloem cells	Remarks
						Thickness × Width	Thickness × Width	
Alpine: Krummholz, windward side, 11,000 ft.	3.2–7.2	25–40	2.4–3.2(1)* 3.2–7.2(2) 3.2–7.2(3)	All cells somewhat palisade-like, larger air spaces than on lee side	105×160	4–5×12–15	10–12×16–19(1) 10–14×12–18(2) (outer cells crushed)	Greater lignification than on lee side
Alpine: Krummholz, lee side, 11,000 ft.	4.8–9.6	25–40	2.4–3.2(1) 3.2–8.4(2) 4.8–8.4(3)	Similar to above but more compact, with smaller air spaces	125×165	4–5×12–15	9–10×15–16(1) 10–15×15–20(2) (outer cells crushed)	.....
Mesic: 9000–10,000 ft.	2.4–7.2	25–30	2.4–4.8(1) 4.8–7.2+(4)	Open, with large spaces	75×62	5×12	9–10×11–15 (outer cells crushed; cambium not evident in older needles)	Lignified in all cells but phloem and epithelial of resin ducts; lignification mainly in middle lamellae
Xeric: Laramie Range	7.2±	30+ (many filled with homogeneous substance)	4.8–9.6	Air spaces relatively small, cell walls thickened and somewhat lignified	90×150	3–4×12–13	13–17×12–20	Lignified in all cells of pericycle and endodermis, xylem, hypodermis, tangential walls of epidermis, and middle lamellae of mesophyll cells
Alpine: on mountainside, over 11,000 ft.	3.2	10–15	1.2–2.4	Very open	75×90	2–3×9–10	6–7×9–11	Pericycle lignified but not endodermis, except slightly in inner tangential and radial walls; hypodermis, epidermis, and mesophyll walls slightly lignified
Current-year needles	3.2–4.8							
One year needles	4.8	15–25	Up to 4.8	Palisade-like cells in rows with spaces between	85×110	3–4×8–11	10–11×11–12	

* Numbers in parentheses indicate age in years.

tats, and the intercellular spaces become filled by cell wall substances. In the mesic situations there are air spaces between many of the hypodermal cells (fig. 13). The resin ducts in the thicker leaves of xeric habitats are larger in diameter than those in the mesic habitats. The intercellular spaces in the chlorenchyma of spruce, as in the fir, are much more evident in the longitudinal sections (fig. 17). The air spaces vary with the habitat, as in the fir, and are larger in the leaves from a mesic habitat. The appearance of a slight infolding in these mesophyll cells of Englemann spruce is made by barlike thickenings on



FIGS. 12–18.—Needles of *Picea engelmanni:* Fig. 12, cross-section, 1 year, mesic habitat. Fig. 13, cross-section, 4 years, mesic. Fig. 14, cross-section, 1 year, xeric. Fig. 15, cross-section, 4 years, xeric. Fig. 16, cross-section, 1 year, high alpine. Fig. 17, longisection, 1 year, mesic. Fig. 18, longisection, 4 years, xeric.

the radial walls between areas of simple pits. These thickenings do not produce the true infoldings characteristic of the pines, except in a few of the mesophyll cells.

The size of the cross-section of the vein is greatest in the leaves from xeric habitats. The phloem has a greater number of cells than the xylem, and the phloem increases more than the xylem by the growth of the cambium. The evidence for cambium growth is also an actual increase in the number of cells of the xylem and phloem. The cambium is not so active as it is in the alpine fir: Lignification is greatest in the xeric habitats, and all cells of the stele are completely lignified, except the phloem—which was never lignified in the leaves examined.

Thickness of the cuticle does not vary as much as on the leaves of the alpine fir. There is a sloughing off of the outer layer (or layers) of cuticle from the older needles and from those of a xeric habitat. The amount of this sloughing was not determined. There was no evidence of this loss of cuticle in the fir but some evidence that it occurs in the spruce. The stomatal pit is overarched by the subsidiary cells of the guard cells more than by the cuticle. The depth of the stomatal pit is practically the same in all habitats.

TABLE 5

PINUS CONTORTA, SIZE OF LEAVES

Habitat	One year old	Two years old	Three years old	Four years old	Av. size for four years
	Length × Width × Thickness (mm.)	Length × Width × Thickness (mm.)	Length × Width × Thickness (mm.)	Length × Width × Thickness (mm.)	Length × Width × Thickness (mm.)
Alpine: near Lake Marie and Medicine Bow Mt., about 11,000 ft.	38 × 1.45 × 0.75	44 × 1.5 × 0.8	40 × 1.4 × 0.7	41 × 1.4 × 0.7	40.7 × 1.4 × 0.74
Mesic: near Univ. of Wyoming Science Camp, 9000–10,000 ft.	47.5 × 1.6 × 0.8	51.2 × 1.6 × 0.6	62 × 1.7 × 0.87	50 × 1.7 × 0.8	52.7 × 1.6 × 0.77
Xeric: dry rocks at the Veedavoo Rocks on Laramie Range	36 × 1.36 × 0.65	39.7 × 1.4 × 0.66	40 × 1.8 × 0.66	35.2 × 1.38 × 0.69	37.7 × 1.48 × 0.66

PINUS CONTORTA LOUD.

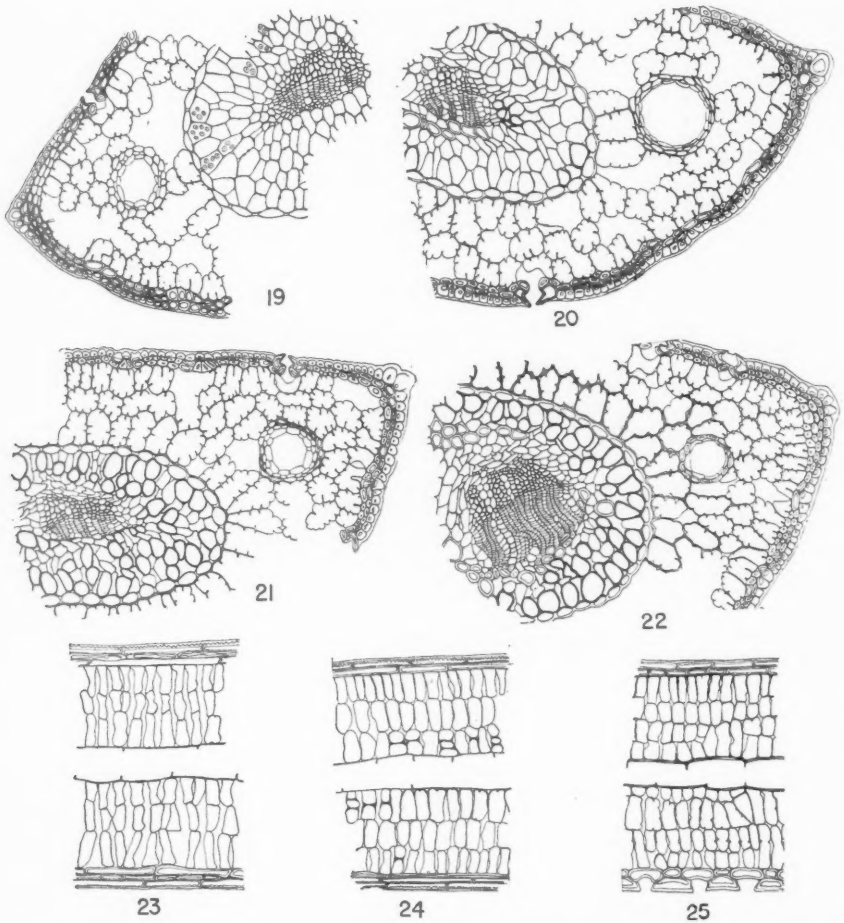
The needles of this pine vary much in size in different years, and there is a noticeable variation in the extremes of habitat. The needles from trees in the most xeric habitat are smallest in all dimensions; those of trees at the highest altitude for the species in this area are next in size; and trees growing in the most mesic habitats have the needles largest in all dimensions (table 5).

The thickness of the epidermal cell walls increases with the age of the needles as in other conifers, the outer walls becoming thicker than the inner. These thickenings are variable in form because of the deep simple pits. There are also simple pits in the hypodermal cell walls, and the walls of these cells become thicker and thicker, until the lumen of the cells may be extremely small in the oldest leaves and in those from a xeric habitat.

The green mesophyll cells in all habitats are infolded. There is an actual infolding of the radial and tangential walls. Longitudinal sections show that these infoldings are of the whole wall and include both primary and secondary walls. Figures 19 and 20 show the degree of in-

folding, the character of the infolded wall, and the greater thickness of the mesophyll cell walls in the older leaves

spaces of greater size than is evident in the cross-sections. As in other species, the largest intercellular spaces



FIGS. 19–25.—Needles of *Pinus contorta:* Fig. 19, cross-section, 1 year, mesic habitat. Fig. 20, cross-section, 8 years, mesic. Fig. 21, cross-section, 1 year, xeric. Fig. 22, cross-section, 8 years, xeric. Fig. 23, longi section from mesophyll, 1 year, mesic. Fig. 24, same, 4 years, mesic. Fig. 25, same, 8 years, xeric.

and in those from the xeric habitats. Although cross-sections of needles from xeric habitats show only very small air spaces, the longitudinal sections of xeromorphic forms show that there are air

are in the leaves from the mesic habitats (figs. 23, 25).

The endodermis is complete and without passage cells. The greatest number of fiber cells occurs in the pericycle of

the leaves that grew in the xeric habitats (fig. 22). The two veins each increase in size after the first year in both extremes of habitat, as shown by the approximate numbers of cells in the xylem and phloem (table 6; figs. 20, 22). This increase in size of the veins is from cambium. The xylem of the needles from the xeric habitat increases by the addition of only a few cells; but the phloem shows a marked increase in number of cells, and the older phloem cells are crushed by this growth. In the leaves from the mesic habitats the xylem increases more than in those from the xeric habitats.

Lignification is greatest in the older needles and in the needles from the xeric habitats. In the leaves from the xeric habitats all cell walls of the pericycle, xylem, the endodermis, hypodermis, and the inner walls of the epidermis become completely lignified. In the green mesophyll the middle lamella, primary wall, and some of the secondary wall are lignified, and near the hypodermis and adjacent to the endodermis the secondary wall becomes completely lignified. The cells of the resin ducts are lignified—except the epithelial cells. The leaves of mesic habitats show lignification first in the middle lamellae and primary walls, then only in narrow lamellae within the secondary wall in the oldest leaves examined.

TABLE 6

PINUS CONTORTA, INTERNAL STRUCTURE OF LEAVES

HABITAT	AV. THICKNESS OF CUTICLE (μ)	AV. DEPTH OF STOMATAL PIT (μ)	AV. THICKNESS OF WALLS OF HYPODERMAL CELLS (μ)	MESOPHYLL IN LONGISECTION (CHLORENCHYMA)	AV. NUMBER OF XYLEM CELLS	APPROX. NO. OF PHLOEM CELLS
					Thickness × Width	Thickness × Width
Mesic: needles full-grown but not year old	2.4	25	1.2	Thin-walled, resin duct walls not thickened, spaces evident	4–5×17–18	6×20
Mesic: needles 5 years old	4.8	25	1.2–3.2	Spaces evident	10×24	20×25*
Mesic: needles 8 years old	4.8	25	1.2–3.2	Cell walls thickened, middle lamellae lignified, spaces evident	7×20	22×20*
Xeric: needles full-grown but not year old	3.2	25	1.2–3.2	Walls slightly thickened, spaces evident	6×17	15–17×16–17*
Xeric: needles 4 years old	4.8	25	2.4–4.8	Intermediate	7×17	17×16*
Xeric: needles 8 years old	4.8–9.6 (at edges of needle)	25	1.2–3.2+	Compact and thick-walled, walls lignified, compact in both trans- and longisection	7×20	22×21*

* Some cells crushed.

Discussion

The cuticle of the leaves of these three species is thickest in the alpine Krummholz and xeric habitats, but in the alpine Krummholz the internal structure of the

leaf is more nearly like that of mesomorphic leaves. In the plants of the alpine meadows around the Krummholz the cuticle is very much thickened also, although the mesophyll of such leaves has relatively very large air spaces, *Polemonium* sp. even having the open mesophyll of an aquatic leaf. The buckbean growing in water at an altitude of 11,000 feet has a surprisingly thick cuticle and the mesophyll of a typical floating leaf. From these observations, it seems probable that the thickness of cuticle varies with the light intensity.

This study did not include any measured data on the physical features of the habitat, or any studies on the relative amounts of water lost. It raises interesting questions, however, that only such data can answer. Why is there a difference, for example, in the thickness of cuticle and internal structure in leaves on opposite sides of the Krummholz, with the more mesomorphic structure occurring in leaves of the windward side?

WEAVER and MORGENSEN (**18**) measured the water lost from conifer seedlings and found that *Pinus ponderosa*, *Pseudotsuga mucronata*, and *Picea engelmanni* lost more water than the seedlings of *Acer glabrum* and *A. saccharinum* per square decimeter of leaf surface. These seedlings grew in the same environment during the time the measurements were made. They also found that the greatest loss of water per day was from 3-year-old seedlings of *Pinus ponderosa* and *P. banksiana*. The 3-year-old seedlings of *Abies grandis* and Douglas fir lost less water per day than the pines. The loss of water from *Picea engelmanni* was less than from the pines but more than that lost by the white fir and Douglas fir.

The lodge-pole pine leaves are the most xeromorphic of the three trees of this study and are similar in general structure to the pines just mentioned. The alpine fir is the most mesomorphic and has leaves most nearly like those of the white fir and Douglas fir used by WEAVER and MORGENSEN. The spruce is the same species. From these data, and from the work of TURRELL (**17**) showing that leaves with the smallest intercellular spaces lose more water per unit of epidermal surface than leaves with large intercellular spaces, it is to be expected that greatest water loss does occur from the leaves of *Pinus contorta*. Does it then follow that the older leaves of these conifers lose more water than the young leaves and that the trees growing in xeric habitats lose more water per unit of epidermal surface than the trees from the more mesic habitats?

Since abscission of the needles of trees in xeric habitats occurs 5–7 years sooner than from trees in mesic habitats, the total amount of water lost must be thereby decreased in trees of xeric habitats. This may therefore be a factor in explaining the continued growth of certain conifers in relatively dry habitats.

It has been generally assumed that the xeromorphic structures described in these leaves from xeric habitats reduce water loss and have a survival value for the plant. The writer is citing these data on water loss measured in relation to intercellular space as a caution not to conclude that the compactness of mesophyll and the other structures typical of plants in xeric habitats are the factors enabling them to survive in those habitats.

Summary

1. In the mesic habitats of the two altitudes in the Medicine Bow Mountains investigated, the needles of *Abies lasiocarpa* were found to be longest, widest, and thinnest; those of *Picea engelmanni* longest and with the smallest

cross-section; and those of *Pinus contorta* larger in all dimensions than those from the xeric habitats of the Laramie Range.

2. Thickness of the cuticle increases with age of the leaves; it is thickest in the xeric habitats and in the bright sunlight of the alpine habitat. Thickness of the walls of the epidermal cells varies in the same way.

3. The stomatal pits tend to be deeper in the older leaves and the leaves from xeric habitats on the fir and spruce; there is little variation in their depth on the pine.

4. The number of cells in the hypodermal sclerenchyma is greater in older leaves, and the greatest number is found in the leaves from xeric habitats.

5. The cells of the mesophyll in all three species are more compact in the older leaves and most compact and with the smallest intercellular spaces in the leaves of trees from xeric habitats. The thickest cell walls and their greatest lignification occur in the leaves from xeric habitats. The resin ducts of largest diameter were found in the leaves from xeric habitats.

6. There is an increase in the size of the veins and in the number of cells of the veins in each species by the growth of cambium. The greatest increase in number occurs in the phloem. Most cambium growth is in the pine.

7. The oldest leaves are found in the trees growing in the mesic habitats. The oldest needles on the wind-blown trees of the high alpine flats are on their lee side.

Eastern Illinois State Teachers College
Charleston, Illinois

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ZONAL STRUCTURE OF THE SHOOT APEX IN ENCEPHALARTOS, BOWENIA, AND MACROZAMIA[1]

MARION A. JOHNSON

Introduction

A recent series of cyto-histogenetic studies on *Ginkgo*, *Cycas*, *Dioon*, and *Microcycas* by FOSTER (**1**, **2**, **3**, **4**, **5**) and on *Zamia* by JOHNSON (**6**) has brought to light a zonal type of meristem heretofore unsuspected. The feature common to these forms is a centrally placed group of cells which has been derived from the superficial layer and its derivatives and which in turn gives rise to a rib meristem and a mantle of flanking meristem. Except for certain variations, to be noted later, this is the fundamental type of architecture observed to date in the shoot apex of cycads. The present paper, while strictly preliminary in scope, owing to the relatively small amount of available material, describes the shoot apex in *Encephalartos*, *Bowenia*, and *Macrozamia*.

MATERIAL AND METHODS.—Special thanks are due Mr. E. R. Thorp of the Botanic Garden, Durban, South Africa; Mr. F. R. Long, Superintendent of Parks, Port Elizabeth, South Africa; and Mr. H. Herre, University of Stellenbosch, Stellenbosch, South Africa, who generously provided Rutgers University with a collection of South African cycads. The following were sacrificed from among the duplicates for histological study: *Encephalartos altensteinii* Lehm.—dormant specimen with caudex 4 cm. in diameter; *E. woodii* Hort.—dormant plant with caudex 11 cm. in diameter; *E. horridus* Lehm.—plant with six new leaves and caudex 28 cm. high and 18 cm. in diameter; *E. lehmanii* Lehm.—bud with one leaf; *E. villosus* Lehm.—plant with trunk 30 cm. high.

[1] Contribution from the Bureau of Biological Research, Rutgers University, New Brunswick, New Jersey.

The late Dr. C. J. Chamberlain kindly placed two seedlings of *Encephalartos frederici-guilielmi* Lehm., three seedlings of *Bowenia serrulata* (Andre) Chamberlain, and three embryos of *Macrozamia moorei* F. Muell. at my disposal. My colleague, Professor M. A. Chrysler, generously contributed the following specimens which had been collected for him by Professor A. A. Lawson in Queensland, Australia: *Bowenia serrulata*, seedling and conebearing female plant with caudex 10 cm. in diameter from which five apices were obtained; *Macrozamia spiralis* Miq., two seedlings with caudex 12–15 mm. in diameter; and *M. corallipes* H., plant with stem 10 cm. in diameter.

The shoot apices were carefully dissected from the surrounding leaf bases of the living specimens and fixed in formalin-acetic-alcohol. All material was dehydrated in tertiary butyl-alcohol, imbedded in paraffin, sectioned at 8 μ, and stained with either tannic acid and iron chloride or safranin and Ehrlich's haematoxylin.

The large size of the shoot apex has made it exceedingly difficult to recognize median longisections with certainty, so that when the term "median" is used it is to be understood that such sections are only approximately median.

Observations

ENCEPHALARTOS

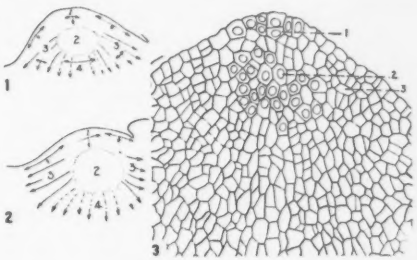
The shoot apex in *Encephalartos* is deeply sunken within a protective armor of cataphylls and leaf bases. It is either broadly cone-shaped or moundlike and may terminate in a plateau about a dozen cells in diameter (figs. 1–5). Measurements of tips taken from median

longisections are: *E. villosus*, 1263 μ in diameter 343 μ from the apex; *E. woodii*, 1170 μ in diameter 436 μ from the apex; *E. horridus*, 858 μ in diameter 171 μ from the apex; *E. lehmanii*, 468 μ in diameter 171 μ from the apex; and *E. frederici-guilielmi*, 530 μ in diameter 187 μ from the apex. The last two measurements are from a bud and seedling, respectively, and consequently give little indication of the size to be expected in adult plants. The others surpass the dimensions recorded for the Florida species of *Zamia* but are considerably smaller than those given by FOSTER (**3, 4, 5**) for *Cycas revoluta* (2–3.2 mm.), *Dioon edule* (1.6–1.9 mm.), and *Microcycas calocoma* (0.5–2 mm.). It seems likely that greater dimensions will be found in specimens of *Encephalartos* with massive trunks, and possibly a 10-foot specimen of *E. woodii* would have a shoot apex approaching that of *C. revoluta* in size.

The shoot apex consists of four zones (figs. 1,2).

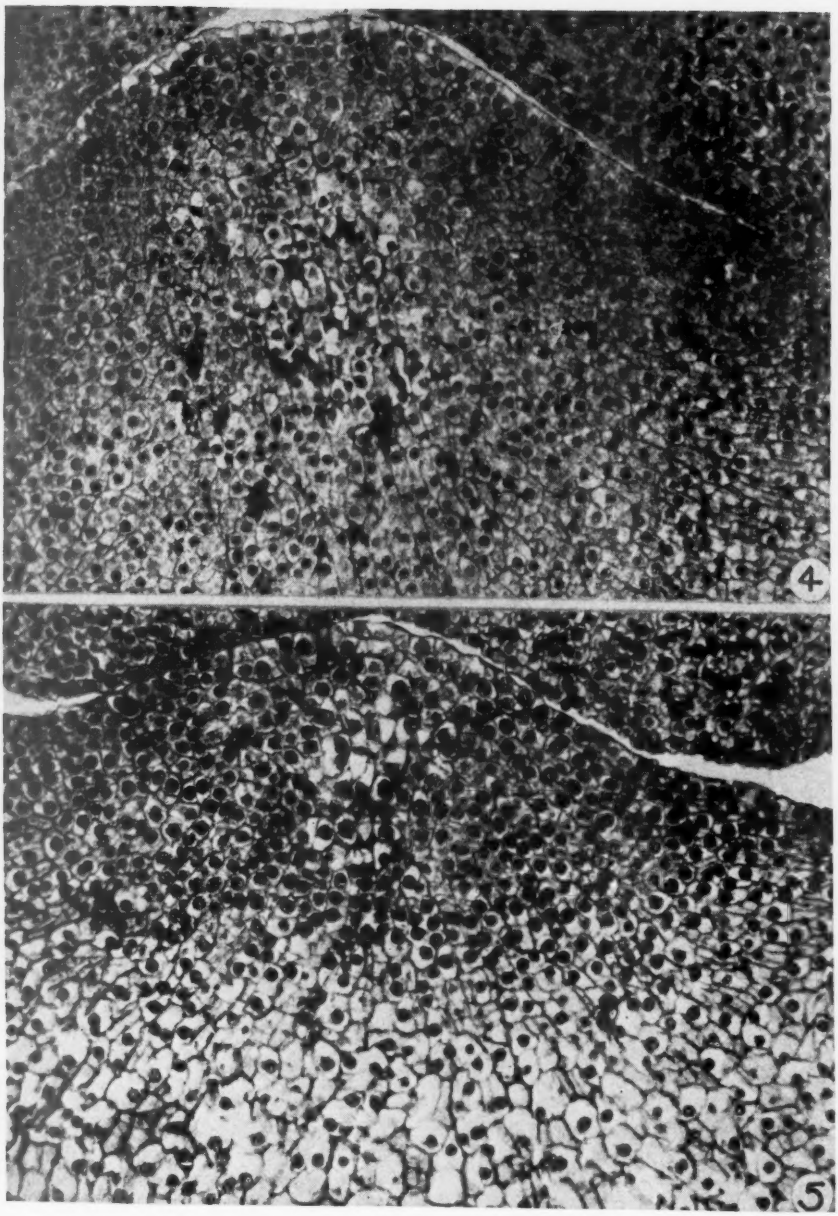
ZONE 1.—The summit of the shoot apex is occupied by a zone of initiation which is generally two to six cells deep and some six to twenty cells in diameter. The superficial layer divides by the insertion of both anticlinal and periclinal walls, thereby contributing to surface growth as well as to the deeper layers within the apex. The latter either build up short chains of cells or complicate the cell pattern by anticlinal and periclinal divisions (figs. 3–5). In dormant tips, the superficial layer may contain conspicuous vacuoles (fig. 4).

ZONE 2 (CENTRAL MOTHER CELLS).—The zone of initiation is underlaid by an area roughly spherical in shape and readily characterized by cellular enlargement and a complex cell pattern. This zone contrasts sharply with the more dense protoplasm and regular alignment of the cells radiating from it (divergent growth). These cells, owing to their position and genetic relationship in the shoot apex, have been appropriately designated the central mother cells by FOSTER (**1, 4**). They originate from the zone of initiation (convergent growth), and—since the transition between the two zones may be gradual—they are not always clearly delimited. In



FIGS. 1–3.—Figs. 1, 2, diagrams showing zonal structure in shoot apex of *E. woodii* and *E. horridus*, respectively. Zone 1 is region of initiation which through periclinal and anticlinal divisions builds up zone 2 (central mother cells). Some contribution is also made to the peripheral meristem (zone 3). Divergent growth from the zone of central mother cells gives rise to the rib meristem (zone 4) and the remainder of peripheral meristem, except for outer part which is derived from the superficial layer. Arrows indicate direction of growth. Fig. 3, median longisection through apex of *E. frederici-guilielmi* (1, zone of initiation; 2, central mother cell zone; 3, peripheral zone). × 215.

massive apices they are more deeply situated than in small ones (figs. 4, 5). It was impossible to determine whether this character is associated with species differentiation or with the size of the apex. The central mother cells are readily plasmolyzed by formalin-acetic-alcohol and are thus rendered conspicuous in stained preparations. Cell division is at random, and intricate cell complexes are built up, giving the region the appearance of a "*massiges Meristem*" (**7**). Genetically related cells in the form of



FIGS. 4, 5.—Fig. 4, *E. lehmanii:* median longisection through shoot apex. Central mother cells show tendency to appear in filaments still inclosed in original mother cell wall; wall thickenings conspicuous; convergent growth toward and divergent growth from zone of central mother cells. × 155. Fig. 5, *E. villosus*, median longisection through shoot apex. Note prominent vacuoles in central mother cells. × 122.

files or irregular blocks are often inclosed in the wall of the original mother cell. Conspicuous wall thickenings are common, especially at the points where several cells touch.

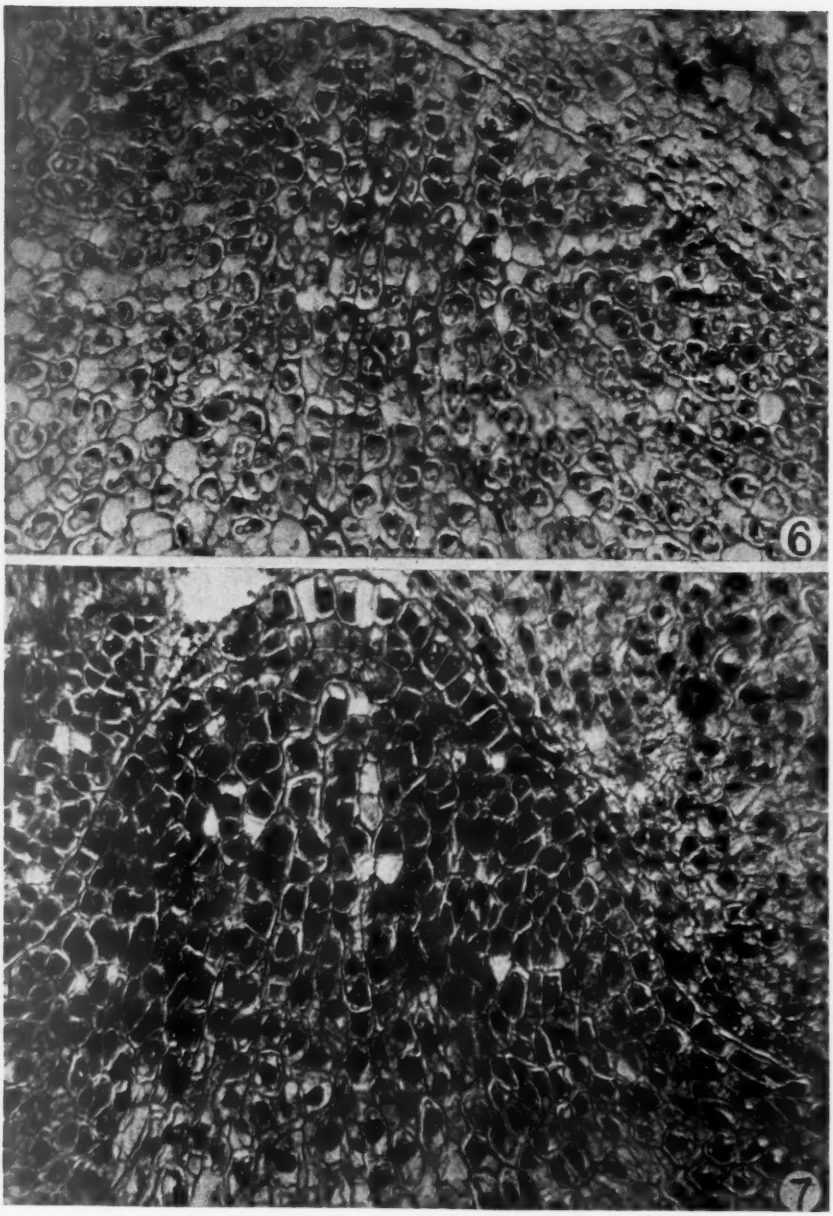
ZONE 3 (PERIPHERAL MERISTEM).—The central mother cells in all the apices examined, regardless of size or species, were imbedded in active meristematic tissue which radiated from them in long files. For the most part these have had their origin in periclinal, anticlinal, and oblique divisions at the edge of the zone of central mother cells. The outer part of the peripheral meristem has been derived from the zone of initiation and the superficial layer and may consist of rows of cells or a complicated irregular pattern. The inner part of this zone is derived from the central mother cells. The greatest activity occurs at the base of the apex, where the leaf primordia originate and where the characteristic cellular arrangement is in files parallel to the surface.

Histologists early called attention to the fact that the shoot apex in cycads is at the bottom of a depression in the stem tip. This condition is largely brought about by a reorientation of division figures and subsequent growth in the peripheral meristem beneath the leaf bases. Divisions parallel to the long files produce a series of cambium-like cells which elevate the periphery of the stem and the leaf bases above the shoot apex. Cortex, vascular tissue, and perhaps some of the pith, mature from the peripheral meristem.

ZONE 4 (RIB MERISTEM).—The long files of cells derived from the base of the central mother cell zone represent a typical rib meristem from which the massive pith differentiates. In the upper portion the cells are densely protoplasmic and division is principally in the transverse plane. Below this level vacuolation becomes noticeable and new walls appear in all planes. The pith now begins an enormous increase in volume, due to both cell division and cell enlargement. The depth of the rib meristem shows some variation, which may possibly be associated with age and periodic growth of the shoot apex. In the seedling of *E. frederici-guilielmi*, elongation of the axis seems to be greater than increase in thickness, and the pith cells are longer than in the other species; also the rib meristem is inconspicuous (fig. 3) and approaches the condition observed by FOSTER (2) in the seedling of *Cycas revoluta*. Whether this condition persists in the adult plant, as it does in *C. revoluta*, cannot be determined until older material is available.

BOWENIA

These observations are based upon longitudinal sections cut from seven apices. The shoot apex is moundlike in shape (fig. 6) and closely invested by several leaf bases. The largest apex measured 1248 μ in diameter 200 μ from the summit. The initiation, central mother cell, peripheral and rib meristem zones are clearly marked and are similar to those described for *Encephalartos* (fig. 6). The central mother cell zone is well developed in seedlings. One specimen, bearing three young leaves and having an axis 10 mm. in diameter, had a shoot apex 545 μ in diameter 45 μ from the tip and a central mother cell zone which was spherical in shape and measured 300 μ in diameter. The contained cells were highly vacuolate and were arranged in a complicated pattern. This pattern seems to be resolved to vertical files of cells in larger apices (fig. 6). The superficial layer contributes less to the pe-



FIGS. 6, 7.—Fig. 6, *Bowenia serrulata*, median longisection showing zonation in shoot apex. × 210. Fig. 7, *Macrozamia spiralis*, seedling apex showing tendency for cells to be arranged in vertical files. Zone of central mother cells poorly developed in seedlings. × 235.

ripheral meristem in seedlings than in older plants.

Macrozamia

Lawson considered the two seedlings studied in this investigation to be *Macrozamia spiralis* (fig. 7), while the large plant (fig. 9) was identified as *M. corallipes*. The latter species is listed as a synonym of *M. spiralis* in Index Kewensis, and according to this opinion the description which follows is therefore for *M. spiralis*. The shoot apex is conical in shape and in the large specimen (fig. 9) measured 624 μ in diameter 265 μ from the tip. The zonation previously observed in cycads is repeated in the shoot apex of *Macrozamia* (fig. 9). It is noticeable, however, that many of the cells tend to be disposed in files parallel to the steep sides of the shoot apex, and while those of the rib and peripheral meristems diverge from the central mother cell zone, yet the angle of divergence is much less than in *Bowenia* or *Encephalartos*. This condition is further emphasized in seedlings (fig. 7) and embryos (fig. 8), where a relatively small group of subapical initials serves as the ultimate origin for all cells except those derived from the superficial layer.

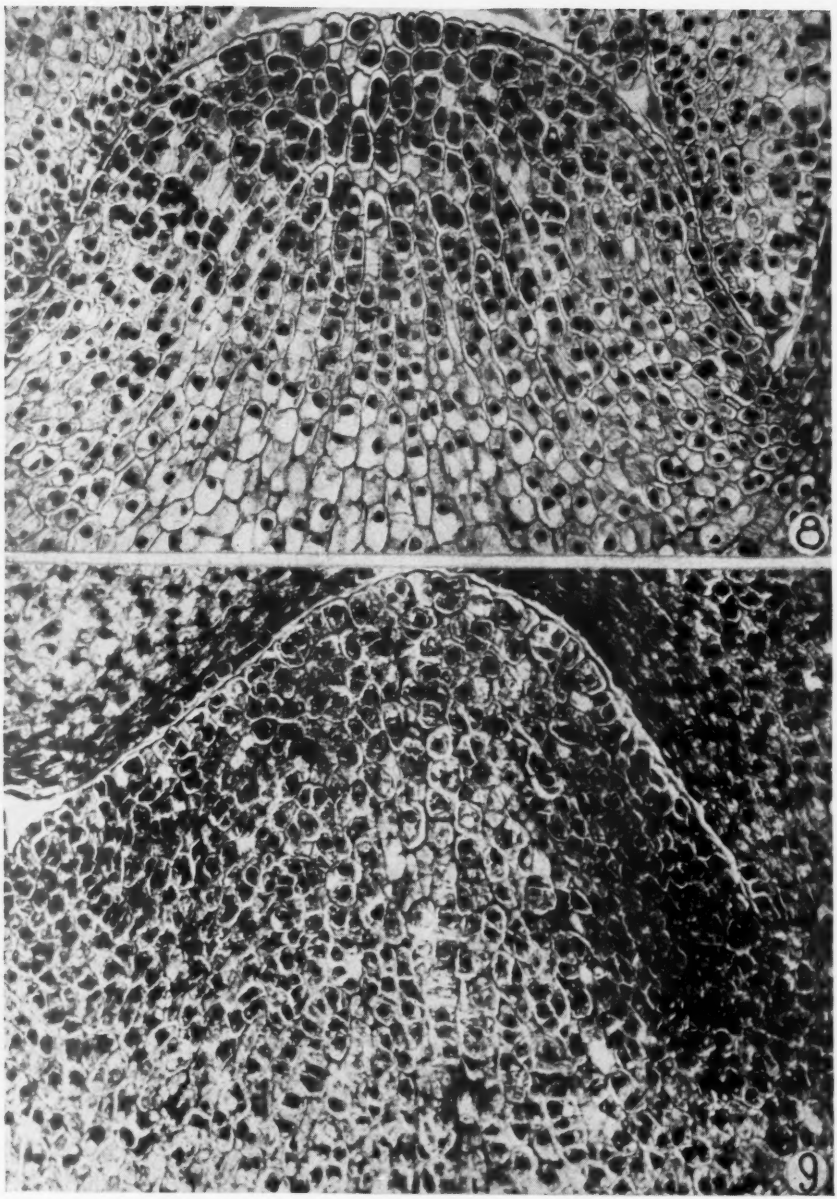
Attention should be called to the enormous shoot apex in the embryo of *M. moorei*, which in the material studied (fig. 8) measured 625 μ in diameter 250 μ from the tip. This is far larger than anything seen even in seedlings of *Cycas revoluta* or *Microcycas calocoma* by Foster (**2, 5**) or of *Zamia* by Johnson (**6**).

Discussion

The zonal structure of the shoot apex, as originally described by Foster (**2**) for *Cycas revoluta*, is now known to occur in *Dioon*, *Microcycas*, *Zamia*, *Encephalartos*, *Bowenia*, and *Macrozamia*. In addition, all have a superficial layer which divides in both periclinal and anticlinal planes; hence cannot be considered as a protoderm. Further agreement is found in the absence of an apical cell. The summit of the shoot apex is occupied by a complex of initials whose number varies with the dimensions of the apex. Those at the exact geometrical center can be compared with a master apical initial only in that they remain meristematic throughout the life of the shoot apex.

The concept of the architecture of the shoot apex in the cycads will not be complete until *Ceratozamia* and *Stangeria*, as well as seedlings from all the genera, have been thoroughly investigated. Our knowledge to date, from seven of the genera, may be briefly summarized as follows. The initial zone is composed of actively dividing cells which converge to the zone of central mother cells. Divisions are anticlinal, periclinal, and oblique. The central mother cell zone, with its generally conspicuously vacuolate cells arranged in a complex pattern, is a region of increase in volume and upon "rejuvenation" at the periphery produces divergent files of cells. The zone of peripheral meristem has a dual origin, in that the outer portion is derived from the superficial layer, while the inner part is from the central mother cells. Leaf primordia, cortex, vascular tissue, and perhaps some of the pith are derived from this zone. A region of rib meristem separates the central mother cells from the pith, except in *Cycas*. Here the demarcation between the maturing pith and the central mother cells is indistinct.

There is a tendency in the seedling of *Macrozamia spiralis* for the cellular arrangement to be in long files parallel to the steep sides of the conical apex. This feature, together with the small number



FIGS. 8, 9.—Median longisections: Fig. 8, *M. moorei*, through apex of embryo; note absence of central mother cell zone and divergent growth from zone of initiation. × 185. Fig. 9, *M. spiralis*, showing zonal structure in shoot apex of adult plant. × 172.

of subapical cells in the initiation zone, is somewhat suggestive of the condition seen in many of the conifers. Further work is necessary, however, before a comparison can be attempted with any other species.

Summary

1. The structure of the shoot apex is described for six species of *Encephalartos* and for one species each of *Bowenia* and *Macrozamia*.

2. The shoot apex consists of four zones. The summit is occupied by a zone of initiation from which growth converges to a zone of central mother cells where increase in volume predominates. Growth is divergent from the central mother cell zone.

3. A peripheral zone, which is derived from the central mother cells and superficial layer, flanks the central mother cell zone. Leaf primordia, cortex, and vascular tissue are derived from this zone.

4. The zone of central mother cells is underlaid by a rib meristem which builds up the massive pith.

DEPARTMENT OF BOTANY
RUTGERS UNIVERSITY
NEW BRUNSWICK, NEW JERSEY

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EFFECTS OF IRON DEFICIENCY ON RESPIRATION OF SUNFLOWER PLANTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY **561**

PAUL R. GLENISTER

Introduction

Iron forms a part of the prosthetic groups of several of the respiratory enzymes found in plant cells—peroxidase, catalase, the cytochromes, and perhaps also cytochrome oxidase. These enzymes have been discussed recently (**4, 16**).

SOMERS, GILBERT, and SHIVE (**14**), working with soybean, found the respiration of iron-deficient less than that of normal plants, as might be expected.

The present work deals with a series of tests in which the respiratory gradients (respiration rates of leaves at various nodes along the stem) of green and of chlorotic sunflower plants were measured to determine whether a depression of the respiration of the leaves of the iron-deficient plants occurred in all the leaves or whether the depression was limited, like the chlorosis, to the younger leaves.

To supplement this study of respiration, the iron gradients (percentages of iron in leaves at various nodes along the stem) of the green and chlorotic sunflower plants were measured to determine (*a*) the extent to which the leaves of the two kinds of plants differed in the amounts of iron they contained and (*b*) the manner in which the iron was distributed among the leaves. With such information, a relationship might be found between iron content, respiration rate, and chlorosis.

Material and methods

During the summer of 1942, sunflower plants (*Helianthus annuus* L.) were grown from seed from a commercial source. The seeds were first removed from the fruit coats and selected for uniformity. They were planted ten per crock in forty 2-gallon crocks which were filled with pure quartz sand. The sand had been cleaned with dilute acid and alkali solutions in the manner described by MINARIK and SHIVE (**8**) and had been washed thoroughly with distilled water. From the day of planting on, the crocks were supplied with nutrient solution (table 1). Half of them received a complete solution and the remainder received one to which no iron salt had been added. One liter of nutrient solution was supplied to each crock every morning. This was usually supplemented with one or more liters of distilled water given during the afternoon, depending on the heat of the day. Germination was complete in 3 days. The seedlings were thinned to one per crock after 2 weeks, in this way securing as uniform a set of plants as possible. Three such sets were grown, but not all the plants were used, because of attacks by aphids, mildew, and other agents.

The carbon-dioxide output of the leaves was measured by using the method and apparatus of MULLISON (**9**). In this method the carbon dioxide produced by the plant material is determined by measuring the changes it causes in the electrical resistance of a definite amount of a solution of standard alkali kept in an absorption tower at a constant temperature. The leaves were inclosed in 500-ml. erlenmeyer flasks with three-holed rubber stoppers and kept there during the tests. One of the holes in the rubber stopper admitted an inlet tube which conducted air from a soda-lime trap to the flask. The outlet tube passed through the second hole and led to the absorption tower. The ends of the inlet and outlet tubes were so placed in the flask that the air had to flow through the body of the flask (and thus over the leaf surface) in going from one tube to the other. The third hole was slit all the way to the side of the stopper, so that the stopper could be opened like a book and closed with the petiole of a leaf going through the third hole in it. A small amount of chewing gum effectively sealed the space between the petiole and the rubber. The flasks were supported by ring stands. By means of this method the simultaneous measurement of the carbon-dioxide output of ten intact leaves on a single plant could be made.

TABLE 1

Composition of complete nutrient solution
(iron salt omitted from minus-iron solution)

Components	Concentration
$Ca(NO_3)_2$	0.006 molar
KH_2PO_4	0.0045
$MgSO_4$	0.0045
B (in H_3BO_3)	0.5 p.p.m.
Mn (in $MnCl_2$)	0.25
Cu (in $CuCl_2$)	0.02
Zn (in $ZnCl_2$)	0.05
Fe (in $FeC_6H_5O_7$)	0.5

The leaves were placed in the flasks at

approximately 12:00 noon, and then kept in the dark during the entire subsequent periods. During the first 20 hours, air circulated through the flasks. Then followed a 4-hour period in which carbon-dioxide-free air passed through the flasks and the pipes of the apparatus. Next the absorption towers were put into the system and the carbon dioxide produced by the leaves collected over a 3-hour period. Fresh weights of the leaves were taken immediately after their removal from the apparatus. Dry weights were taken after the leaves had been in an oven kept at 97° C. for 24 hours.

The iron contents of individual leaves were measured by the official colorimetric method (1), using a ferric-alum solution as standard, as described by SNELL and SNELL (13).

Seventeen green and twenty chlorotic plants, possessing twelve to twenty-five leaves each, were tested for respiration gradients. Ten or eleven leaves per plant were tested for carbon-dioxide production; then all the leaves were analyzed for iron.

Results

Symptoms of iron deficiency were noticeable in the plants of the minus-iron treatment after the third week of growth. At this time the leaves of the fifth, sixth, and seventh nodes were pale green or yellow in color, with the principal veins of a darker green. No death of tissue occurred with the chlorosis. As these leaves matured they gradually assumed a greener color. The leaves which appeared at succeeding nodes behaved similarly; they developed as chlorotic leaves and became greener some time after expansion. As a result of this later greening of leaves originally chlorotic, the younger leaves were the only chlorotic ones of the iron-deficient plants.

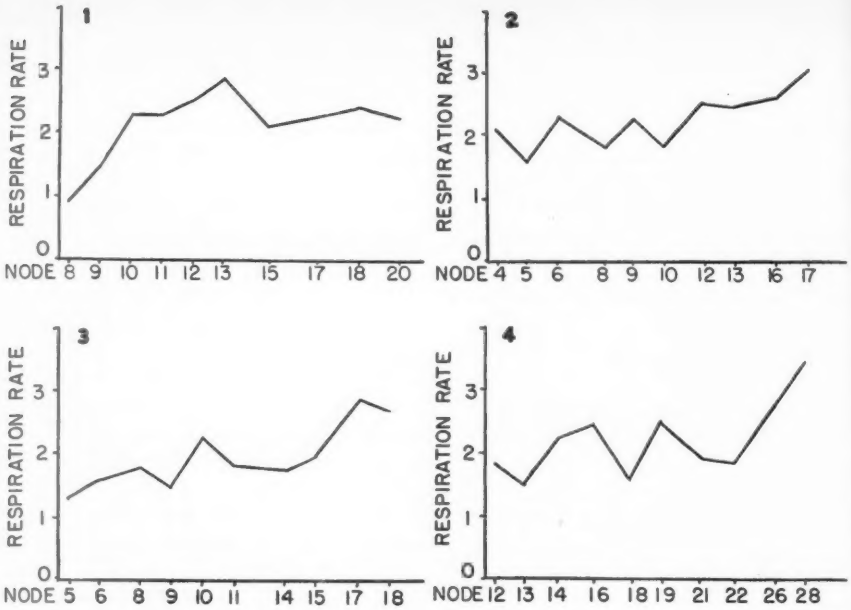
There were no noticeable size differences between the two kinds of plants.

The respiratory gradients of four representative plants of the plus-iron treatment and four of the minus-iron treatment are presented in figures 1–8. The respiration rates (expressed as milligrams of carbon dioxide per gram of dry weight per hour) are plotted along the vertical axes and the numbers of the nodes at which the leaves occurred are plotted along the horizontal axes. The node number was determined by designating the cotyledonary node number 1, and continuing the count from this point to the top of the plant.

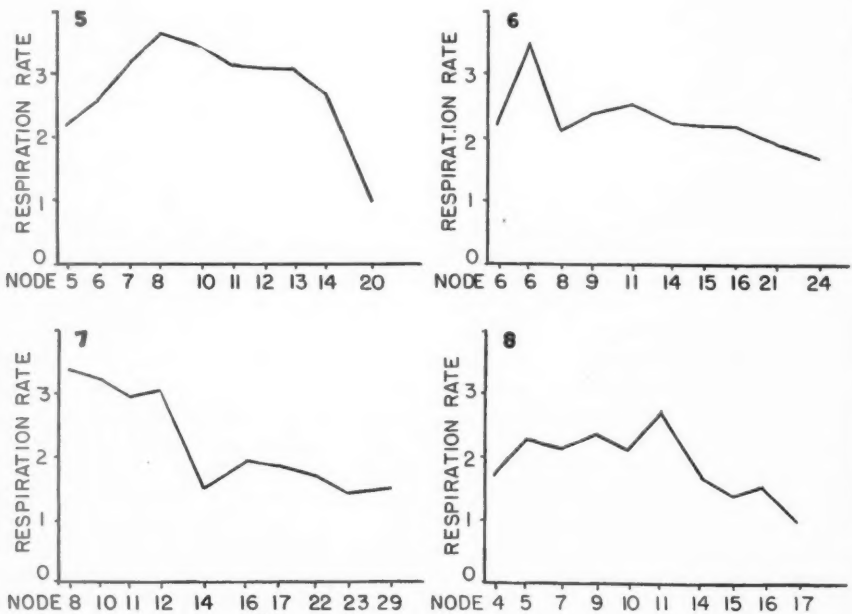
The respiratory gradients for the plants of the plus-iron treatment indicate that the younger leaves were respiring more than the older ones. Such differences are apparently normal, having been reported for other plants (**2, 3, 6, 10, 11, 12**). The respiratory gradients for the iron-deficient plants indicate that the younger and chlorotic leaves were respiring as much as or less than the older leaves. These gradients are abnormal and indicate a depression of the respiration of the younger chlorotic leaves.

Leaves at adjacent nodes on the same plant may differ greatly in respiration rate. This causes the respiratory gradients to be irregular but does not affect the general trend of the gradients of the two kinds of plants.

Figure 9 is based on the iron gradients of a plant of each treatment, both plants being the same age. These gradients illustrate four findings: (*a*) the lower older leaves of both kinds of plants contain about ten times as much iron as the younger upper leaves; (*b*) the gradients of iron content from older to younger leaves are in gradually decreasing order; (*c*) the leaves of the plant grown with complete nutrient solution contain (with



FIGS. 1–4.—Plus-iron plants: Fig. 1, plant 15, set 1. Fig. 2, plant 6, set 2. Fig. 3, plant 8, set 2. Fig. 4, plant 12, set 2.



FIGS. 5–8.—Iron-deficient plants: Fig. 5, plant 13, set 1. Fig. 6, plant 16, set 1. Fig. 7, plant 18, set 1. Fig. 8, plant 5, set 2.

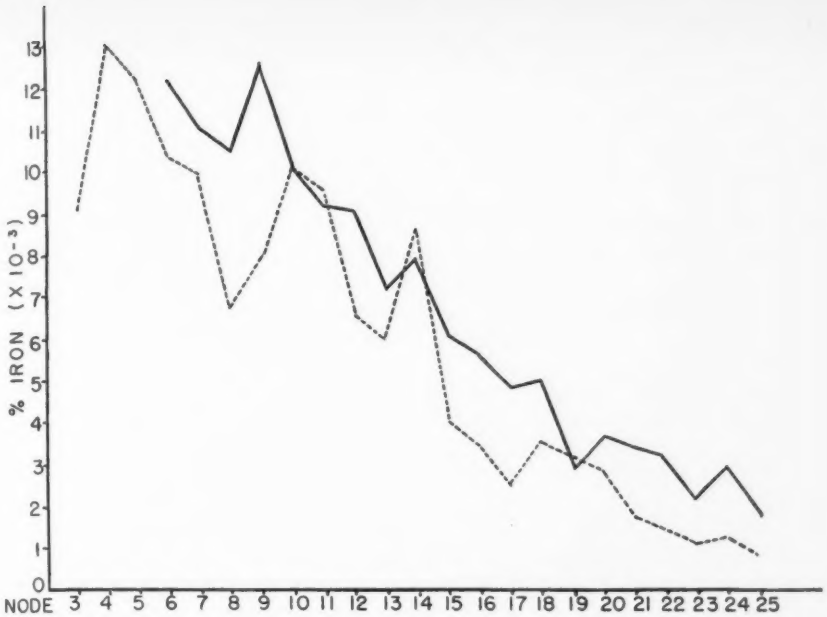


FIG. 9.—Iron gradients in two plants of equal age. Solid line, plus-iron plant; broken line, iron-deficient plant.

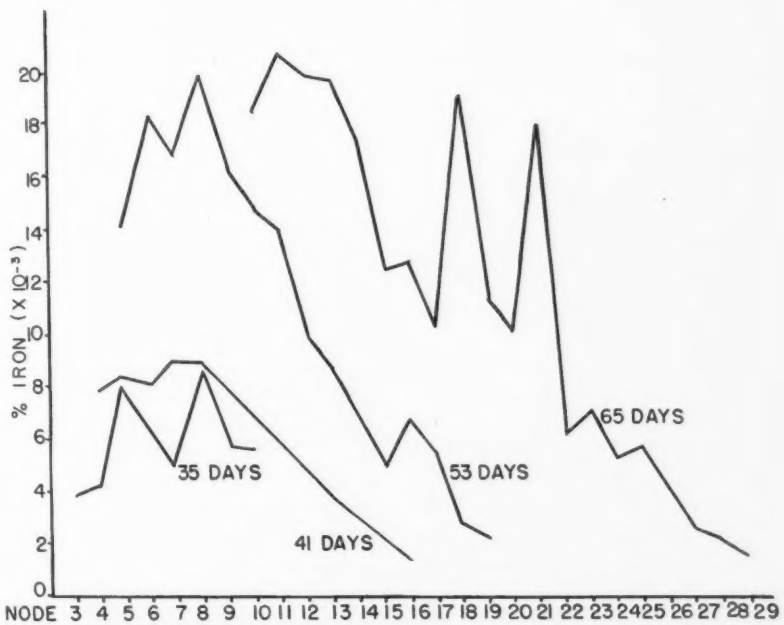


FIG. 10.—Total iron content gradients of plus-iron plants of different ages

a few exceptions) from slightly more than once to more than twice as much iron as the leaves of the iron-deficient plant; (*d*) this difference is greatest in the younger leaves.

Both the chlorosis and the depression of respiration of the iron-deficient plants occur in the leaves with the greatest deficiency in iron—the younger leaves. Figure 10 presents the iron gradients of four plants of similar treatment but of different age. It shows that the iron content of leaves at corresponding nodes is proportional to the age of the plants compared. The older a plant is, the more iron is contained in its leaves.

TABLE 2

Node	Green weight (gm.)	H_2O (%)	Dry matter (%)	Fe ($\times 10^{-3}$) (%)	Respiration rate
	Typical plus-iron plant				
5	1.346	84.62	15.38	12.04	1.3
6	1.819	86.36	13.64	16.98	1.6
8	3.219	87.60	12.40	14.95	1.8
9	3.940	87.60	12.40	9.79	1.4
10	4.583	86.82	13.18	10.28	2.2
11	4.157	87.01	12.99	8.93	1.8
14	3.214	85.96	14.04	7.52	1.7
15	3.454	85.72	14.28	5.94	1.9
18	2.016	85.06	14.94	8.71	2.9
19	1.673	84.93	15.07	6.86	2.9
	Typical iron-deficient plant				
4	1.629	85.87	14.13	11.28	1.8
5	1.749	87.53	12.47	11.93	2.3
7	4.202	88.83	11.17	8.07	2.2
9	4.662	89.44	10.56	10.12	2.6
10	4.744	86.81	13.19	8.10	2.4
11	4.781	88.72	11.28	7.75	2.8
14	5.776	88.22	11.78	4.65	1.7
15	4.446	87.69	12.31	3.78	1.4
16	3.296	88.53	11.47	2.76	1.4
17	2.834	88.14	11.86	3.16	1.0

Table 2 gives the data for green weight, water content, dry-matter content, iron content, and respiration rate for a representative plant of each treatment. These data show that the differences in iron supply did not have a great influence on growth of the plants as reflected by these measurements. Direct comparisons of this sort are impossible, because corresponding nodes were not used in every plant and because the plants were of different ages when tested for respiration.

Discussion

The effects resulting from iron deficiency, both chlorosis effects and respiration effects, are localized in the younger leaves. This indicates that iron is not transferred from the older to the younger parts, and that a continuous supply of iron must be available if the younger leaves are to have normal color and normal respiration rate.

The gradients of iron content shown in figure 9 support this interpretation. The gradient for the iron-deficient plant follows the same pattern as that of the plant grown with complete nutrient. If relocation of iron from older to younger leaves had occurred in the iron-deficient plant, its gradient would have been quite different, especially in the region of the older leaves.

It appears to be a general phenomenon that the deficiency symptoms of nonreutilized elements occur primarily in the younger leaves. Chief among such elements are calcium, boron, manganese, copper, and iron. Elements which can undergo reutilization, such as nitrogen, phosphorus, potassium, and magnesium, have deficiency symptoms which occur primarily on the older leaves (**5**, **7**).

In the present work the chlorotic leaves of the iron-deficient plants were found to contain some iron. Lack of it

must have been relative rather than absolute. The problem is therefore one of suggesting a reason for the inactivity of the iron in these leaves. Two mechanisms of inactivation of iron have been suggested by previous investigators.

The first is that the iron is precipitated in the conducting elements because of the high pH of the plant sap. Such precipitated iron would presumably be of no value to the plant. Chlorosis of this type is usually caused by a high calcium content of the soil (**2**).

The second mechanism of iron inactivation is that involving the iron-manganese ratio. It has been shown by solution culture methods that an iron-deficiency chlorosis will occur if iron is not maintained in the plant in the ratio of 2:1 with manganese (**14**). The postulated mechanism (**15**) is briefly as follows. Active iron is ferrous iron, and it tends to be kept in that state by the reducing mechanisms of the tissues. Manganese, because it has a greater oxidation potential than iron, will—when present in excess in relation to iron—tend to keep the iron in the inactive ferric state.

In the present study the first mechanism of inactivation can hardly apply. The concentration of calcium in the nutrient solution was not excessive, and the frequent applications of distilled water which were applied would have kept the calcium from accumulating to an excessive concentration.

The second mechanism of inactivation seems to be the more likely one in the present case. The ratio of iron to manganese in the nutrient solution applied to the iron-deficient plants was far below 2:1. A difficulty with this second mechanism, however, is its premise that active iron is in the ferrous state. While such an assumption may apply to the function of iron in chlorophyll synthesis, it can hardly apply to its function in respiration. The iron in the cytochrome system functions by way of a cyclic change from the ferric to the ferrous condition. It is the same in the enzyme catalase. Peroxidase occurs and functions only in the ferric condition. It seems more likely that the observed depression of respiration may have been caused by the action of the manganese in preventing or hindering the cyclic valence changes of the iron-containing enzymes in which such changes occur, rather than by the removal of iron from an active ferrous state.

Summary

1. Sunflower plants were grown in sand culture with nutrient solution. One group had iron salt as ferric citrate added to the nutrient solution; the other had no iron salt added.

2. The plants of the minus-iron treatment developed the typical chlorosis symptoms of iron deficiency; the controls were of normal color.

3. Respiration rate was depressed in the young chlorotic leaves of the iron-deficient plants.

4. The chlorotic leaves of the iron-deficient plants contained only half as much iron as comparable leaves of control plants. No relocation of iron from older to younger leaves occurred in the iron-deficient plants. In both the gradients were of decreasing iron content in going from the bottom to the top of the plants.

5. The iron contents of the leaves of a given plant of either treatment were found to be related to the ages of the leaves. The older contained more iron than the younger leaves.

Department of Botany
University of Chicago
Chicago, Illinois

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EMBRYOLOGY OF PASPALUM DILATATUM[1]

HUGH W. BENNETT

Introduction

The increasing importance of *Paspalum dilatatum* (Poir.) as a pasture plant warrants more detailed studies of its cytology, morphology, and life history. Information from such studies will have a direct application to the development of improved strains through plant breeding and might reveal the causes of failures of attempted hybridization and the low percentage of seed set. Seed production is the factor limiting the more widespread use of this grass. The object of this study was to trace the developmental morphology of the embryo and to determine not only the manner but also the order and rate of development of the various parts. The normal development of the embryo would serve as a starting point for investigations of abnormalities in *P. dilatatum* and in other species of the genus.

Previous studies have dealt with determination of the homologies of various portions of the embryo. Relatively little attention has been given to the important stages of development.

According to VAN TIEGHEM (16), the earliest description of the embryo of grasses is that of *Avena* and *Triticum* by MALPIGHI in 1687. He recognized the structures now known as the scutellum, epiblast, and coleoptile. The homologies of these organs are still a controversial subject, as shown by views presented in the more recent papers by AVERY (1), HOWART (4), PERCIVAL (11), and MERRY (9). Detailed views of certain phases of

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embryo morphology have been prepared by VAN TIEGHEM (16), BRUNS (3), KENNEDY (6), and AVERY (2). MCCALL (8) has presented a critical review of the extensive literature and a summary of the various views pertaining to gross morphology and homologies.

Early studies on development were based mainly on observations of whole embryos. NORNER (10) studied the early divisions of the zygote and proembryo of *Hordeum*, *Avena*, *Triticum*, and *Secale* by dissecting out whole embryos and mounting them in glycerin. The oldest stages he figured were just beginning to show organ differentiation. He attempted to classify the arrangement of the cells according to the manner in which they divided.

SOUÈGES (14) traced the parts of the fully developed embryo of *Poa annua* to the tier of cells in the sixteen-celled proembryo. He maintained that the parts of the embryo are determined at least as early as the sixteen-celled stage. He also indicated that there was a definite arrangement of the cells and a regular sequence of cell divisions in the development from the fertilized egg.

RANDOLPH (12) has given a complete description of embryogeny for *Zea*. He found that there was no definite arrangement of the cells and no regular sequence of divisions in the early stages. The sectors of the proembryo in the initial stages of development were so rapidly obscured that it was impossible to determine or predict which sector or sectors gave rise to the root and shoot regions of the plumule-radicle axis. He considered temperature relations as an important factor influencing the rate of development.

MERRY (9) has also given a complete description of the embryogeny of *Hordeum sativum* from the time of fertilization to maturity of the seed. He found no arrangement of cells or sequence of cell divisions in the proembryo. He shows that the embryos of *Hordeum* varying one day in age have recognizable morphological differences.

LA RUE and AVERY (7) showed a rapid development of the embryo of *Zizania aquatica*, the mature embryo reaching a length of 10 mm. in 19 days. In that species the cotyledon of the embryo extends the entire length of the fruit and often extends around the end of the caryopsis. Growth and development of the Z. *aquatica* embryo are dependent on association with tissues of the plant and it cannot be grown in artificial culture.

Apparently the only reference concerning the embryo of the genus *Paspalum* is that of *P. pubiflorum glabrum* by KENNEDY (6), based on the mature embryo. It is described as having a large radicle; its scutellum-bundle is inserted some distance from the plumule; there is no epiblast; and one foliage leaf is present.

MATERIAL AND METHODS.—Material for embryological studies was obtained from potted plants of strain 56–17 growing in a shaded greenhouse. Samples were collected at specific intervals after pollination. It was necessary to open each spikelet and remove the growing seed or infertile ovule with fine-pointed forceps because of the indurate nature of the lemma and palea. Counts showed that 72% of the ovules were dried and thus not fertile.

The material was killed in Craf and Allen-Bouin (13) fluids. Suction was applied to material younger than 6 days to give total immersion. Material was imbedded in paraffin through an n-butyl-alcohol series. Paraffin sections 10–15 μ in thickness were stained with Delafield's haematoxylin; some sections were stained in safranin fast green.

Observations

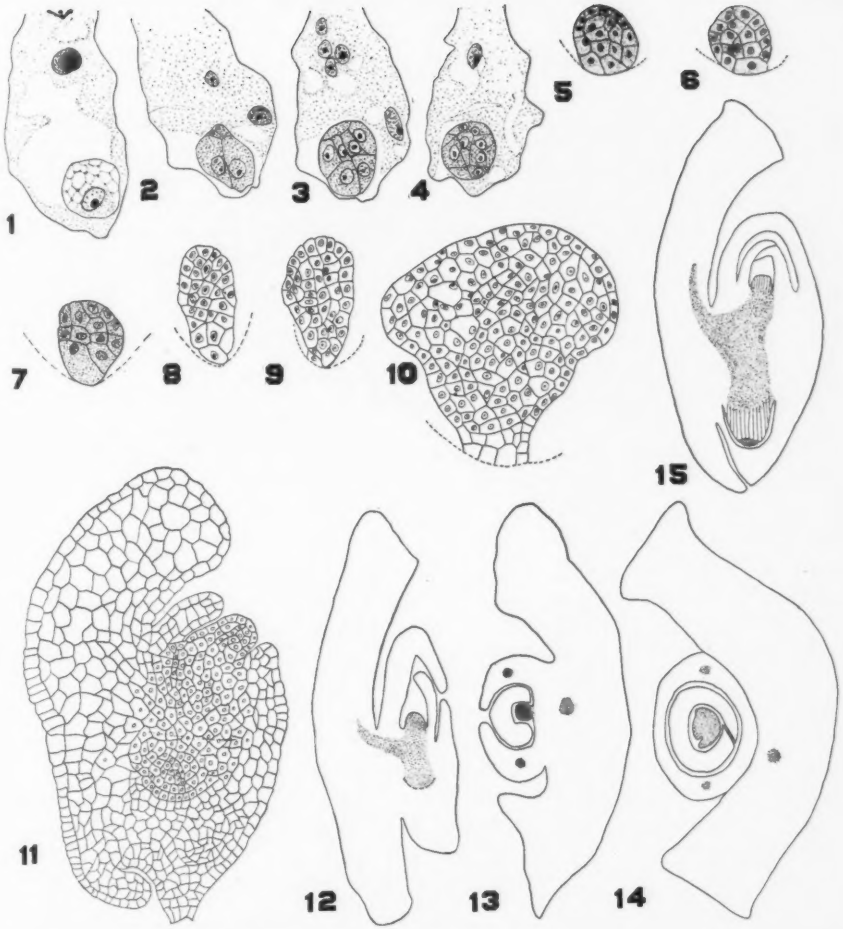
Pollen grains germinate very soon after anther dehiscence. The rate of pollen-tube growth within the style has not been determined, owing to difficulty in clearing the feathery, much-branched stigmas. Pollen-tube growth is probably rapid, as the stigmas appear to be dry and dead within 2 hours after anther dehiscence. During periods of rainy weather, with the accompanying high humidity, some stigmas are alive and not wilted the day after anthesis. Fresh pollen on such stigmas has not induced fertilization, however, indicating that this occurs the day of anthesis, possibly very soon after anther dehiscence. Delay in collecting pollen has resulted in a lowered pollen germination on artificial media.

The time interval between pollination and fertilization, under average summer conditions, is between 8 and 12 hours. Zygotes have been found 8 hours after pollination (fig. 1) and small proembryos after 12 hours (figs. 2–4). Twelve hours after pollination the proembryo consists of two to eight cells and is approximately 25–29 μ in length. The zygote and primary endosperm nucleus undergo division very soon after fusion is completed. The two-celled embryo consists of a small apical cell and a much larger basal cell. There are rapid free nuclear divisions of the endosperm (fig. 3). Twenty-four hours after pollination the proembryo becomes rounded, ranges 33–46 μ in length, and consists of from eight to sixteen cells (fig. 5). The proembryo begins to lengthen at 36 hours. The apical cells continue to be smaller than the basal cells (fig. 6). The endosperm continues free nuclear.

Differentiation of organs begins during the period from 60 to 72 hours (figs. 7, 8). An increase in the rate of cell division in a posterior subapical region and in a zone near the apex of the anterior side results in a slight protuberance on each side. This evidently is a preliminary step in the initiation of the coleoptile-radicle axis and of the posterior lobe (left side of fig. 9). The embryo is approximately the same size as 12 hours earlier. Four days after pollination, rapid meristematic activity on the posterior face of the embryo results in enlargement of the posterior lobe, which becomes the basal portion of the scutellum. The suspensor elongates and becomes sharply delimited from the body of the embryo (fig. 10).

Differentiation and growth are extremely rapid during the fourth and fifth days (figs. 10, 11). The coleoptile-radicle axis is 160 μ long and only slightly oblique to the longitudinal axis of the embryo, which is 148 μ long. The scutellum enlarges by expansion of the posterior lobe upward and downward. The growing point of the plumule and the coleoptile primordia are very distinct (fig. 11). The coleoptile is formed as a ridge of tissue which nearly surrounds the central growing point. The definite arclike orientation of cells in the lower portion of the axis marks the beginning of differentiation of the coleorhiza. The embryo at this stage is approximately 324 μ long.

On the sixth day, the ridge of tissue forming the coleoptile has developed more rapidly above than below the central meristem and has formed a sheathing structure that envelops the stem tip. The foliage leaf arises below the stem meristem as a ridge of tissue similar to and opposite that of the coleoptile primordium (fig. 13). Pronounced differentiation of the scutellar trace has taken place. The planes of continued cell division within the lower part of the axis are oriented to produce a dome-shaped zone, which delimits the coleorhiza and pri-



FIGS. 1–15.—Fig. 1, zygote 8 hours after pollination. Figs. 2–4, proembryos 12 hours after pollination. Fig. 5, proembryo 24 hours. Fig. 6, proembryo beginning to lengthen 36 hours after pollination. Fig. 7, proembryo 48 hours. Fig. 8, proembryo 60 hours. Fig. 9, embryo 72 hours. Fig. 10, 4-day embryo; posterior lobe considerably enlarged. Fig. 11, embryo 5 days after pollination, showing distinct coleoptile primordium, plumule growing point, and organization of radicle tip. Fig. 12, embryo 6 days after pollination, showing rapid growth of coleoptile and distinct seedling leaf primordia. Fig. 13, cross-section of 6-day embryo, showing seedling leaf and coleoptile around plumule growing point. Fig. 14, cross-section of 14-day embryo, showing seedling leaf and initiation of another leaf primordium. Fig. 15, mature embryo, 18 days after pollination.

mary root. The scutellum has lengthened to 629 μ (fig. 12).

The side of the coleoptile next to the scutellum grows faster than that on the anterior side, so that by the seventh day the coleoptile is almost closed, leaving the coleoptile pore on the anterior side. Differentiation of the root cap has begun. The embryo has now reached a length of 684 μ. By the eighth day the leaf primordium has begun to overlap the growing point. The coleorhiza is clearly organized and was differentiated from the lower part of the embryo by the development of the primary root primordium. The scutellar trace is one-half the length of the plumule by the eleventh day. The embryo attains practically mature size in 14 days, having a scutellum length of approximately 780 μ, a plumule-radicle axis of 530–590 μ, and a thickness of approximately 250 μ (fig. 14).

The seedling leaf continues to lengthen and is curved around the growing point at 14 days. Another leaf primordium has begun to differentiate as a small protuberance above the stem meristem directly opposite the first leaf. This second leaf fails to develop further, and there is only one seedling leaf in the mature embryo (fig. 15).

Discussion

Following division of the zygote, the subsequent planes of cell division in the proembryo are irregular, and apparently no special significance can be attached to the sequence of cell division or to the arrangement of cells in the early development of the embryo. This is in agreement with the findings of RANDOLPH (12) with corn and of MERRY (9) with barley and indicates that the factors controlling growth of the proembryo for the first 60 hours affect the embryo as a whole, rather than definite cells. SOUÈGES (14) held that parts of the embryo are already determined in particular cells in the sixteen-cell embryo. NORNER (10) also attempted to classify the arrangement of cells according to the manner in which they divided.

The proembryo is almost round for the first 60 hours after pollination and is comparable to the same stages of growth in *Eragrostis cilianensis* (All.) Link., as described by STOVER (15). During these stages, the only perceptible differentiation is the difference in the size of the apical and basal cells. During this period, growth of the proembryo is limited chiefly to the apical region and may suggest a gradient from the apex to the base of some factors which may control the rate of division of the cells. The appearance of smaller cells in certain particularly highly meristematic parts of the embryo at 3 and 4 days may indicate localization of similar factors in those regions.

The development of the embryo from initiation of the axis (3 days) to maturity follows that of the ordinary grass type of embryo as described by LA RUE and AVERY (7), RANDOLPH (12), MERRY (9), STOVER (15), and summarized by MCCALL (8).

The mature embryo of *P. dilatatum* differs from the embryo of many grasses in that it has only one foliage leaf and no epiblast. It differs from the embryo of *P. pubiflorum glabrum* (6) in that the axis is more oblique to the main body of the embryo.

The embryo of *P. dilatatum* is mature 14–18 days after pollination. This is relatively rapid in comparison with development of the embryo in maize, which requires 45 days (12), and barley, which requires 35 days (9). The rate of maturity is slightly faster than that found for *Zizania aquatica* by LA RUE and AVERY (7), who regarded the 19-day-old

embryo to be mature but the seed "unripe." The fastest rate of development of a grass embryo is that reported by JENKINS (5) for *Poa annua*, in which 82% of the caryopses were mature 14 days after pollination.

Some evidence of cleavage polyembryony has been found in *P. dilatatum*, which might well be one cause of the low yield of viable seed.

Summary

1. The time interval between pollination and fertilization of *P. dilatatum*, under average summer conditions, is 8–12 hours.

2. The endosperm nuclei are free for the first 2 days. There is no consistent arrangement or zonation of cells in the young embryo.

3. Initiation of the coleoptile-radicle axis begins in 60–72 hours.

4. Development of the embryo is extremely rapid from the fourth to the eighth day and is structurally complete in 14–18 days.

5. The mature embryo has one foliage leaf and a distinct radicle with a root cap.

6. There is no epiblast.

The writer wishes to express his appreciation to Dr. J. E. SASS for his valuable suggestions during the study of this phase of the problem.

MISSISSIPPI STATE COLLEGE
STATE COLLEGE, MISSISSIPPI

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GROWTH AND DEVELOPMENT IN RANGE GRASSES. IV. PHOTOPERIODIC RESPONSES IN TWELVE GEOGRAPHIC STRAINS OF SIDE-OATS GRAMA[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 562

CHARLES E. OLMSTED

Introduction

Many recent and some older investigations have emphasized the characteristic growth and habits of strains of native species obtained from different locations in their respective distributional areas (often involving altitudinal or latitudinal gradients) when they are grown side by side under nursery or greenhouse conditions (**3, 7, 13, 18, 19, 25, 26, 27, 29, 32, 33, 34**). Irrespective of the original causes of the genotypic differentiation which has brought about such diversity, it has usually resulted in a high degree of physiological and often of morphological adaptation to the environment in which a strain (ecotype) is now native, as compared with its responses in the native environments of related strains (**7, 29, 34**). Such differentiation and adaptation have long been known for agronomic and horticultural crops and some forest trees and are the basis for much of the work in plant breeding, variety testing, studies of acclimatization, etc.

Much interest has been shown in the differing photoperiodic responses of various strains and varieties of many species of cultivated plants (**2, 4, 5, 8, 9, 10, 11, 14, 20, 24, 36**). The range of phenotypic expression induced by different photoperiods is of obvious applied significance in the seasonal and latitudinal adaptation of any particular strain. Possible variation in photoperiodic responses among strains of native species has been largely ignored, or has been studied only in a limited number of strains, often not systematically selected (**1, 2, 14, 15, 30, 31**). Consequently, it seemed that an investigation of the photoperiodic responses of different latitudinal strains of a widely ranging native species (with consequent differences in range of natural photoperiods in the different localities) might yield data of considerable interest.

Earlier work (**23**) had demonstrated that a strain of side-oats grama grass (*Bouteloua curtipendula* (Michx.) Torr.) from southern Arizona consisted largely of short-day plants with a critical photoperiod between 14 and 14½ hours. Since northern strains flower on much longer natural photoperiods, it was thought that this species might exhibit considerable differentiation in photoperiodic responses or requirements. Strains from different latitudes, grown together in a number of places (**13, 16, 19, 26, 27**), have shown morphological, physiological, and cytological diversity, correlated in part with latitude of origin.

Side-oats grama occurs over much of eastern, central, and southwestern United States, extending into Canada on the north (southern Saskatchewan), southward into Mexico, and in South America to Argentina (**16, 17**). In the United States it is of greatest importance in the western part of the prairie region and at certain altitudes in the Southwest, where it is a valuable forage plant, for both hay and grazing. It has shown considerable promise in the artificial or natural reseeding of drought-stricken, overgrazed, and eroded land, and it is thought by some to be the most promis-

[1] This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

ing grama for domestication (**16, 18, 27, 35**). A knowledge of the photoperiodic responses of some of its strains should be of value in: (*a*) helping to explain the observed differences among them when grown together under nursery conditions (**13, 16, 19, 26, 27**); (*b*) interpreting the factors affecting their vegetative and reproductive habits during the growing season; (*c*) assisting practical plant breeders to select or breed strains for reseeding purposes by indicating the possible restrictions that photoperiodic sensitivity would impose. The investigation might also be of interest from the standpoint of the evolution of adaptive physiological responses in general and of the operation of natural selection as the range of a species is extended (**7, 22, 33**). The latter has implications for plant geography, especially with respect to environmental factors limiting migration and possible changes in the limiting values of factors for particular species in the course of time.

Experiments were initiated in the spring of 1942 at the University of Chicago and have been continued since. The present paper is a survey of the photoperiodic responses of twelve strains from different latitudes during 1942 and 1943. Additional work on some of these strains is in progress. Related work by LAVIN (**21**) on geographical strains of blue grama (*Bouteloua gracilis* (H.B. K.) Lag.) has not yet been published.

Material, methods, and environmental conditions

Seed of twelve strains of side-oats grama (fig. 1) was obtained from various sources (table 1). Strains were designated by number as indicated. The latitudinal range from San Antonio, Texas, to Cannonball, North Dakota, is approximately 17°. The periods between sunrise and sunset at these two stations at the summer solstice are approximately 14 and 16 hours, respectively (effective photoperiods probably near 15 and 17 hours, allowing for dawn and twilight). The rate of increase to or decrease from these maxima to or from the nearly equal period of slightly over 12 hours from sunrise to sunset at both stations at the equinoxes (effective photoperiod under 13 hours) is obviously greater at the northern station.

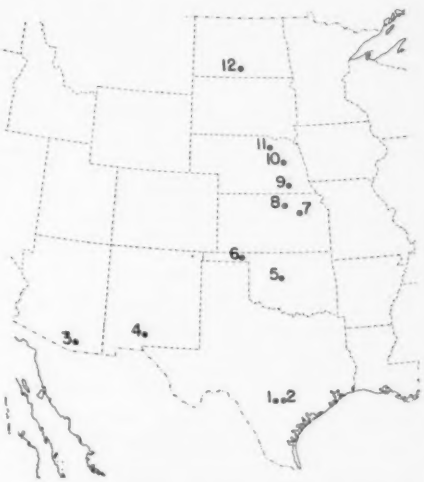


FIG. 1.—Geographical sources of strains numbered as shown.

In the 1942 experiment, 225 8-inch unglazed clay pots were evenly filled and packed with a silt loam soil of prairie origin (near Chicago) and of medium fertility. They were divided into five equal series (45 pots in each). On April 11, uniform caryopses were planted 1 cm. deep at evenly spaced intervals at the rate of 30–45 per pot, as governed by previous germination tests. Thirty-five pots in each series were planted with strains nos. 1, 4, 5, 7, 9, 11, and 12 (five pots per strain) and ten pots in each

series with strains nos. 2, 3, 6, 8, and 10 (two pots per strain).

Immediately after the prompt and uniform germination (all seedlings to emerge appeared by April 15), the five series were placed on photoperiods of 9, 13, 16, and 20 hours, and natural day-length. Two series on two movable trucks received natural daylight for 9 hours between 8:00 and 5:00 P.M. (CDT) but were rolled into ventilated light-proof sheds for the remainder of the 24 hours. One of these two series was given 4 hours of artificial light from 5:00 to 9:00 P.M., to provide a 13-hour photoperiod. The third series on a stationary truck in the same room received supplementary illumination after sunset to furnish a 16-hour photoperiod throughout the season. The fourth and fifth series were placed in separate greenhouse rooms. The latter was on natural day (N series) and the fourth received artificial illumination after sunset to provide a 20-hour photoperiod. The intensity of the supplementary light, from 200-watt Mazda lamps mounted in individual reflectors over the different series, varied from 100 to 180 foot-candles (Weston sunlight meter) at average foliage height, depending upon pot position and plant height.

TABLE 1

SOURCE OF SEED OF TWELVE STRAINS OF SIDE-OATS GRAMA

Strain no.	Point of origin of strain	Approximate latitude	Harvested from	Year harvested	Received from	Accession no. of agency
12	Cannonball, North Dakota	46½	SCS Nursery, Mandan, North Dakota	1941	R. W. Carpenter, SCS, Mandan, North Dakota	NDG 204–41
11	Holt County, Nebraska	42½	SCS Nursery, North Platte, Nebraska	1941	E. C. Conard, SCS, Lincoln, Nebraska	373–41
10	Platte County, Nebraska	41½	SCS Nursery, North Platte, Nebraska	1941	E. C. Conard, SCS, Lincoln, Nebraska	2409–41
9	Jefferson County, Nebraska	40	Field	?	J. E. Weaver, Univ. of Nebraska, Lincoln, Nebraska	.....
8	Cloud County, Kansas	39½	Field	1940	E. C. Conard, SCS, Lincoln, Nebraska	3177–40
7	Alma, Kansas	39	Field on farm of August Feyh	?	Donald R. Cornelius, SCS, Manhattan, Kansas	KG1823
6	Beaver County, Oklahoma	37	Field	1941?	D. A. Savage, BPI, Woodward, Oklahoma	.....
5	El Reno, Oklahoma	35½	SCS Nursery, Manhattan, Kansas	?	Donald R. Cornelius, SCS, Manhattan, Kansas	KG1234
4	Las Cruces, New Mexico	32½	Field	1941	Anton Berkman, Texas Coll. of Mines, El Paso, from U.S. Forest Service	.....
3	Santa Rita Exp. Range, Arizona	32	Field	1938	R. A. Darrow, Univ. of Arizona, from U.S. Forest Service	.....
2	San Antonio, Texas	29½	SCS Nursery, San Antonio, Texas	1941	D. E. Griffith, SCS, San Antonio, Texas	T-3846 (1–41)
1	Bexar County, Texas	29½	SCS Nursery, San Antonio, Texas	1941	D. E. Griffith, SCS, San Antonio, Texas	T-3841 (1–41)

Responses on Chicago natural day-length may be usefully compared with behavior under field conditions. A 13-hour photoperiod is near or below the lower limit of the photoperiodic range on which any of the strains flower under

natural conditions, while 16 hours of light per day is somewhat below the maximum to which the northern strain is naturally subjected during part of its growth period. Light periods of 9 and 20 hours were used to determine whether critical photoperiods occur above or below the other values. It should be remembered that plants in the 9-, 13-, 16-, and 20-hour series were subjected to daily photoperiods of constant length throughout the season, while in the N series they were under the influence of the changing natural photoperiods. The maximum natural photoperiod at Chicago at the solstice is 15 hours and 14 minutes (sunrise to sunset).

Plants were thinned to ten per pot during the first 2 months of growth. During the summer, efforts were made to maintain similar favorable moisture, temperature, and natural light intensity values among the various series. Practically all plants were harvested the fourth week in September by clipping the tops at approximately 2.5 cm. above the soil surface. One pot of each strain under each treatment was then washed out for examination of roots and rhizomes. The remaining pots were placed on natural photoperiod during the winter of 1942–43, except as indicated in succeeding paragraphs, and left in the same greenhouses as during the summer. During October vegetative growth was resumed, the three southernmost strains (1–3) soon reaching average foliage heights of 25–30 cm. on the shorter days of autumn, while growth of the more northern strains was proportionately less with increase in latitude of origin, with a tendency to develop a rosette habit, irrespective of previous treatment. There was little internodal elongation in any strain at this time, and no further flowering occurred until May and June of 1943.

In November the temperature was reduced in the house containing the series previously on 20-hour photoperiod, and values ranging from 35° to 55° F. were maintained throughout the winter and early spring. In late April, 1943, they again approached those of 60°–75° which had been maintained in the other two houses over winter. Plants in the cool greenhouse were practically dormant from November to May. The foliage which had been produced in October died back in November and December to within 2.5 cm. of the soil level. These plants resumed growth slowly about May 1, and by June 27 all strains had mostly attained average foliage heights of approximately 30 cm., although no flowering and little internodal elongation occurred. Some plants of strain no. 1 not clipped in September resumed growth at the tips of the stems formed in the summer of 1942. This 20-hour series of 1942 was left on natural photoperiod during the summer of 1943 and was designated 20-N.

The N series of 1942 was left in a warm greenhouse over winter, and because of bench position it received natural light of lower intensity than did the other four series during the winter months. The plants also became dormant in November and December, most of them dying back to the ground in a manner comparable with those which had been on 20-hour photoperiod in the summer. They resumed growth slowly, the southern strains first, in early April. By May 25 the three southern strains had average foliage heights of about 30 cm., while the northern strains were still rosettes. No culm elongation had occurred by this date, when this series (N-N) was placed with the 20-N series.

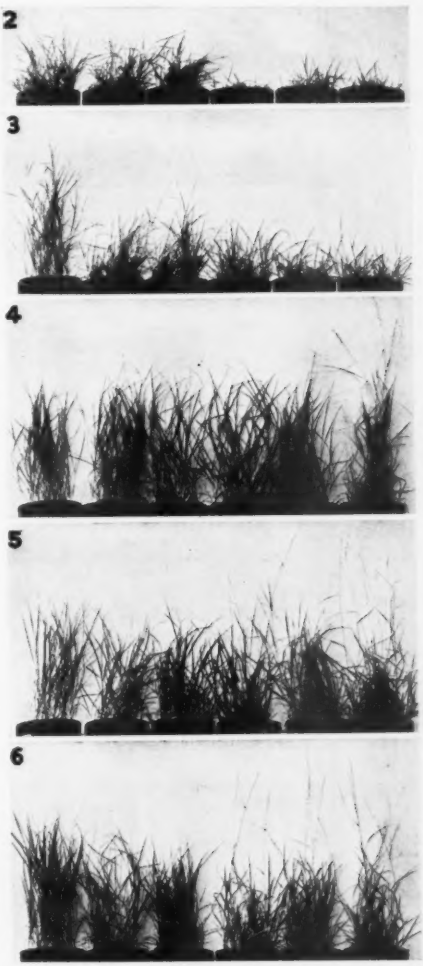
Plants which had been on 9-, 13-, and 16-hour photoperiods until late September, 1942, were left in a warm greenhouse with the maximum natural light intensity possible in the greenhouses at Chicago in the winter months. They also mostly became dormant in November and December, but not quite so markedly as the other two series. Most of the foliage produced in October by the three southern strains remained green. Their further growth was limited after December until about March 15, when growth was resumed—both from new tillers and from those of the preceding autumn. By May 21 these three southern strains averaged about 40 cm. tall, although there had been little culm elongation. The apparent depth of dormancy in the more northern strains over winter increased with latitude of origin. In the North Dakota strain (12) practically all foliage died, so that the only visible green tissue in December was located in stem bases or small tillers in the crown within 2 inches of the soil level. Some plants of this strain were then given supplementary illumination (values comparable with those given above) to provide a 20-hour photoperiod during January, 1943. Growth was resumed, and within 30 days the average foliage height was about 25 cm. No other plants of any strain on natural photoperiod grew at this time. This new growth died back to the ground when the pots were placed on 8 hours of natural light per day in February. The other plants of the nine northern strains in these three series resumed growth during the latter part of March and early April, height growth being more rapid in the southern strains, so that for about a month there was a decreasing gradient in height from southern to northern strains. By mid-May size differences were less pronounced, all plants ranging 30–45 cm. tall. There was some culm elongation in the northern strains, and a few plants of strains 10 and 12 showed exserted inflorescences on May 15. These apparently had developed in response to the increasing length of the natural photoperiod.

On May 21 all plants of the 9-, 13-, and 16-hour series of 1942 were clipped to within 3 cm. of the soil level. These pots were then redistributed among four series on 9-, 13-, 16-hour, and natural photoperiods during the summer of 1943. One pot each of strains 1, 4, 5, 7, 9, 11, and 12 from each of the 9-, 13-, and 16-hour series of 1942 was available for each of the four series of 1943, so that a total of thirty plants of each of these strains was placed on each new treatment. The one pot available for strains 2, 3, 6, 8, and 10 from each of the 9-, 13-, and 16-hour series of 1942 was placed on a similar photoperiod in 1943, but none were available for the N series. In 1943, because of "blackout" regulations, all plants received supplementary illumination in light-proof sheds. Thirteen- and 16-hour photoperiods consisted of 9 hours of natural daylight with 4 and 7 hours, respectively, of supplementary illumination varying in intensity from 100 to 180 foot-candles.

Six major series were thus run in the summer of 1943. One series (N-N) consisted of forty plants each of strains 1, 4, 5, 7, 9, 11, and 12, and ten plants each of the other five strains which had grown on natural photoperiod in 1942, had been clipped in September, and were then left on natural photoperiod for the ensuing months in a warm greenhouse. The second series (20-N) consisted of the above number of plants of each strain, grown on 20-hour photoperiod in 1942 before clipping in September, on

natural photoperiod after clipping, and subjected to cool conditions during the winter of 1942–43. These two series were placed together on May 25, 1943, and both were allowed to grow on natural photoperiod thereafter. The other four series were as outlined in the preceding paragraph, with thirty plants each of strains 1, 4, 5, 7, 9, 11, and 12 on each of 9-, 13-, 16-hour, and natural photoperiods; and ten plants each of strains 2, 3, 6, 8, and 10 on each of the 9-, 13-, and 16-hour photoperiods. In these four series all plants had been clipped in September, 1942, and were again clipped on May 21, 1943. After this date an effort was made to maintain similar environments for all six series, except for duration of the light periods. Development and sequence of flowering were followed until late October, 1943.

Supplementary illumination was continued in 1943, after the conclusion of the major experiments, to provide some plants with a 16-hour photoperiod during the ensuing winter months, while others were placed on natural photoperiod. All were in the same warm greenhouse after November 1. Some foliage of plants of all strains remained green when provided with supplementary light, although new growth was limited after December and no culm elongation or flowering occurred in any strains during the winter months. In those plants not receiving supplementary illumination after October, dormancy developed comparable with that of the preceding year. Some of the foliage of the southern strains remained green but without new growth, while foliage of the northern strains died back. In late December all plants were supplied supplementary illumination to provide a 16-hour photoperiod. They grew slowly in January, and by mid-February new foliage had developed on all strains to lengths of 15–20 cm., but no flowering had occurred by late March. ROGLER (**26**), from experiments in North Dakota and Minnesota, reported that strains of this species



FIGS. 2–6.—Ten plants of side-oats grama per pot on August 6, 1942, 118 days after germination. Left to right: strains 1, 4, 5, 9, 11, 12, in latitudinal sequence from south to north; grown on photoperiods of: fig. 2, 9 hours; fig. 3, 13 hours; fig. 4, 16 hours; fig. 5, 20 hours; fig. 6, Chicago natural daylength.

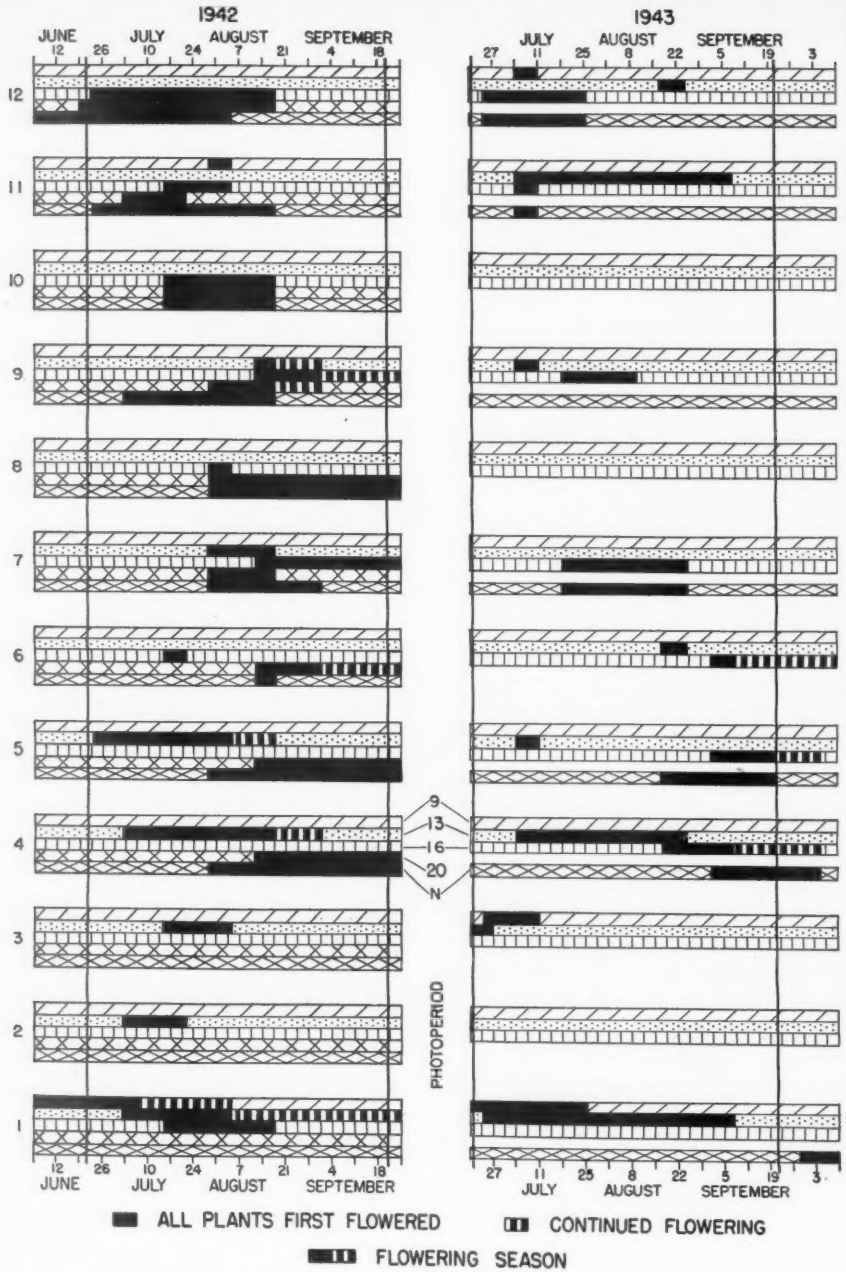


FIG. 7.—Flowering seasons of twelve strains of side-oats grama in 1942 and 1943 grown on photoperiods (hours) as indicated. Germination on April 11, 1942; plants clipped May 21, 1943. Solid bars show period in which individual plants first exserted inflorescences; broken bars show period of continued flowering by one or more plants.

from a wide latitudinal range tended to become dormant in the fall, even at high temperatures, but he did not suggest any explanation.

Because of failure to flower and limited growth in the greenhouses during winter at Chicago, critical experiments on photoperiodic responses of this species are possible here only during late spring and summer. Conclusions must therefore be based chiefly on the responses observed during the first and second summers of growth. Development during the second summer was apparently affected to some degree by over-wintering conditions and whether the plants were clipped previously during May (figs. 7, 8; table 3). Those plants kept in a warm greenhouse under the highest possible natural light intensity during the winter months of 1942–43 showed less profound dormancy than did the others. They renewed growth earlier in the spring and grew more vigorously before they were clipped on May 21 than did those on other winter treatments. Winter chilling is apparently unnecessary for vigorous growth the following year, while the appearance of inflorescences on two of the northern strains by mid-May without winter chilling suggests that cold temperatures are not needed for the initiation of reproductive activity. SHEPHERD (**28**), however, reported that a strain from Hays, Kansas, grew more vigorously there in the winter months from sods brought into the greenhouse on December 30 than from green sods brought in on October 13 which had become "dormant and sere" by November 18, although watered. The latter sods renewed growth in 21–69 days after treatment with potassium thiocyanate on November 18, and in 54–69 days without chemical treatment on natural and 16-hour photoperiods, respectively. None of SHEPHERD's plants flowered during the winter months. His plants on 16-hour photoperiod grew more vigorously than those on natural daylength. No freezing or chemical treatments to break dormancy in the winter months have been tried by the writer, but the results with supplemental illumination in autumn and winter months suggest that dormancy of these strains in warm greenhouses may in part be induced by a complex of external conditions, among which decreasing photoperiod and decreasing light intensity are important. Under

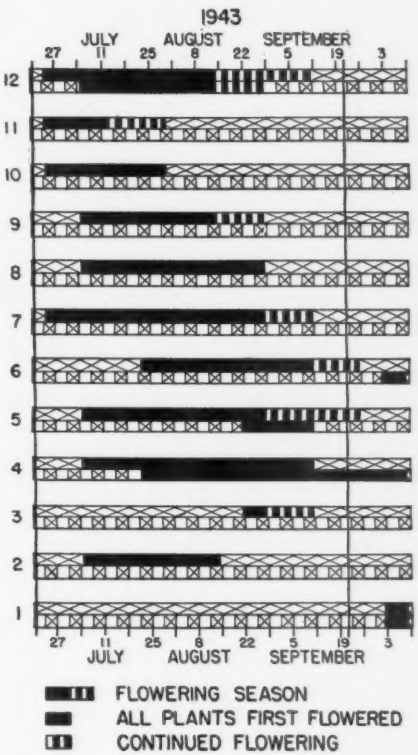


FIG. 8.—Flowering seasons of twelve strains of side-oats grama in 1943 on Chicago natural photoperiod. Germination on April 11, 1942; plants not clipped in 1943. Upper and lower of each pair of bars are N-N and 20-N series, respectively.

field conditions in their native environments, cool temperatures and drought are probably equally if not more important, although undoubtedly decreasing photoperiod has an effect. ROGLER (26) at Mandan, North Dakota, and HOPKINS (19) at Hays, Kansas, working with the same species, have both reported that in nursery-grown plants from a wide geographical range there was progressive delay in maturity—and subsequent dormancy—with decrease in latitude of strain origin. The southern strains were regularly injured by frost at these stations before they were dormant, while the strains from latitudes near or north of these stations became semidormant several weeks before the first frost. The results reported in this paper suggest that these responses may have been due largely to the differing effects of the decreasing photoperiods on the various strains used by these investigators.

There is certainly need for more investigation on the factors which induce and break dormancy and which prevent flowering in this species in the greenhouse in the winter months. Enough has been done, however, to suggest that winter dormancy or lack of it probably is of little importance in affecting photoperiodic responses in the summer, if the plants resume growth early in the spring—as they did in all except the 20-N series. The difference in flowering seasons and vigor of flowering between the N-N and 20-N series in 1943 (fig. 8; table 3) can be attributed in part to the delay in renewal of growth of the latter, owing to its subjection to cool temperatures in April, as well as to the different treatments over winter.

The photoperiodic treatments of 1942 seemed to have relatively little effect on responses the following year. The responses were similar in the three pots of each of the seven strains in the 9-, 13-, 16-hour, and N series of 1943 which had been grown on three separate treatments in 1942. It seemed justifiable, therefore, to average the 1943 data within each group of three pots. These results are in contrast with TINCKER'S (31) observations on several species of grasses. He reported considerable effect of different photoperiodic treatments one season upon growth and flowering the following year.

The four series placed on 9-, 13-, 16-hour, and N photoperiods on May 21, 1943, were clipped on that date in order to remove any developing inflorescences and possibly to prevent aftereffects which could be attributed to photoperiodic induction (6) of some of the strains during their exposure to the increasing natural photoperiods of 12 or more hours when they resumed growth in March and April. A lack of much aftereffect of previous photoperiod when plants were so clipped at the time of reciprocal transfers among different photoperiods has been reported (23) for strain 3. The May clipping may not have been effective in removing all inflorescence primordia. Two plants of strain 12 showing several emergent inflorescences on May 21 had each again produced one visible but weak inflorescence 44 days later (on July 11) on the 9-hour photoperiod (fig. 7; table 2). Since these were the only plants of the nine northern strains flowering in the 9-hour series in 1943, these inflorescences may already have been initiated before the plants were placed on this photoperiod and escaped clipping owing to proximity to the soil level. It has not yet been possible to work out the developmental morphology of all the strains or the minimum length of time required from initiation to exsertion

of inflorescence (12). It is known that, after initiation, flower buds in certain chrysanthemums may persist on photoperiods unfavorable originally to initiation. Their further development may be retarded, or completely inhibited, until the proper photoperiod is provided (2). In other species, primordia may be initiated under a wide range of photoperiods but will develop into visible flowers only over a more limited range (5, 30). In the Arizona strain of side-oats grama, inflorescences may be initiated and exserted in less than 4 weeks of growth after clipping when on appropriate photoperiod (23). They may also be rapidly initiated on elongated vegetative culms. Whether this is true of the other strains is not known, although exserted inflorescences appeared at 30 and 34 days after clipping on strains 1 and 12 on 9- and 16-hour photoperiods, respectively (table 2).

Clipping in May probably caused some reduction in the vigor of growth and flowering of plants so treated. This is apparent to a limited extent in comparing the 1943 N series so clipped with the unclipped N-N series (figs. 7, 8; table 3). The length of the flowering season of certain strains in the former series was probably shortened also, since the clipping undoubtedly destroyed some inflorescences which otherwise would have been exserted in late May or June. This should be borne in mind in comparing the three N series of 1943 (N, N-N, 20-N) which had not received identical treatment at any time until late May, 1943.

It was not possible to use as many or as large pots for these experiments as for those previously reported (23) or to transplant as originally planned. Consequently, there was considerable competition among the ten individuals in each pot, and growth and flowering were restricted, especially in the second year, although the conditions were no more severe than the species encounters in nature. Plants of the same strain and treatment were not so uniform in size as in previous experiments, and undoubtedly some individuals failed to flower which would have done so if less crowded. While the quantitative data are therefore less decisive, and the variation among individuals in each strain on each treatment cannot be ascribed chiefly to genetic differences within the populations represented, yet the qualitative differences—both vegetative and flowering—among strains and treatments seem sufficiently clear-cut to point to definite conclusions as to the nature of the photoperiodic responses of the various strains.

Results

Time required for first flowering

The beginning and progress of flowering were measured by the exsertion of inflorescences from the surrounding leaf sheaths. Time and materials did not permit dissection methods, which would have shown initiation of inflorescences in the apical meristems (12). For the purposes of this investigation, however, the effects of different photoperiods in inducing, allowing, or preventing development of fertile inflorescences are of equal significance with their effects on the initiation of floral primordia.

Flowering of young plants, 1942.—Table 2 shows the approximate number of days after germination when the first inflorescence appeared in each strain on each treatment. It was formed on the primary axis except in three cases. While these data are based on only one plant

from each population, they are indicative of the responses of the various strains. If the columns are read both vertically and horizontally, the failure or progressive delay in onset of flowering among the various strains in one treatment thus shown, or of the several treatments of one strain, suggests strongly that the southern strains 1, 2, and 3 are either short-day or intermediate (2) in their photoperiodic responses, while the northern strains are long-day. Data for plants on Chicago natural photoperiod indicate, as do many other data, that the three southern strains respond in a different manner from the nine northern ones. Although there was a more or less progressive increase in the delay of first flowering of the nine northern strains with decrease in latitude of origin, some plants of all nine strains apparently are able to initiate and mature inflorescences on the long natural photoperiods of June and July at Chicago. Indeed, some plants of all nine strains did so on a 20-hour photoperiod, although with increasing delay with decrease in latitude of origin. The three southern strains, however, had failed to produce inflorescences when clipped on September 23, on either Chi-

TABLE 2

NUMBER OF DAYS UNTIL FIRST FLOWERING,* AFTER GERMINATION ON APRIL 11, 1942, OR AFTER CLIPPING ON MAY 21, 1943, FOR STRAINS AND PHOTOPERIODS (IN HOURS) AS INDICATED

STRAIN NO.	PHOTOPERIODS (1942)					PHOTOPERIODS (1943)			
	9	13	16	20	N	9	13	16	N
12	.....	.....	73	69	55	44‡	88‡	34	34
11	109†	.....	95	81	73	....	44	44	44
10	.....	.....	95	95	95	....	.....	.....	X§
9	.....	123†	123	109	81	....	44	59	.....
8	.....	.....	109	109	109	....	.....	.....	X§
7	.....	109†	123	109	109	....	.....	59	59
6	.....	.....	95	123	123	....	88	103	X§
5	.....	73	.....	123	109	....	44	103	88
4	.....	81	.....	123	109	....	44	88	103
3	.....	95	.....	.....	.....	34	31	.....	X§
2	.....	81	.....	.....	.....	....	.....	.....	X§
1	55	81	95	.....	.....	30	34	193	130

* Measured by exsertion of inflorescence from surrounding leaf sheath.

† In 1942 data, where so indicated, inflorescences appeared only on tillers; in all other 1942 data they were exserted first on primary axes.

‡ Inflorescences weak and sterile; primordia possibly initiated before clipping, since plants were flowering when clipped.

§ No plants of this strain on this photoperiod.

TABLE 3

PERCENTAGES OF PLANTS FLOWERING* BY SEPTEMBER 23, 1942 AND BY OCTOBER 19, 1943

STRAIN NO.	GERMINATION APRIL 11, 1942 PHOTOPERIODS (1942)					CLIPPED MAY 21, 1943 PHOTOPERIODS (1943)				NOT CLIPPED 1943	
	9	13	16	20	N	9	13	16	N	N-N	20-N
12	0	0	60	52	78	7†	3†	30	13	58	15
11	2	0	16	12	40	0	10	3	3	10	0
10	0	0	15	25	50	0	0	0	X‡	50	0
9	0	4	8	14	22	0	7	13	0	23	0
8	0	0	5	10	20	0	0	0	X‡	70	0
7	0	4	12	6	8	0	0	10	7	28	0
6	0	0	5	15	5	0	10	10	X‡	20	10
5	0	8	0	6	12	0	7	20	10	13	5
4	0	10	0	12	10	0	7	17	10	10	15
3	0	15	0	0	0	20	10	0	X‡	10†	0
2	0	35	0	0	0	0	0	0	X‡	40†	0
1	66	96	2	0	0	67	93	0§	13	13	3

* One or more inflorescences per plant exserted above surrounding leaf sheaths.

† Inflorescences weak and relatively sterile.

‡ No plants of this strain on this photoperiod.

§ Seven % on December 1, 1943.

cago natural or 20-hour photoperiods, or in the 16-hour series—except for one plant of strain 1. There is apparently a critical photoperiod above which plants of these three strains will not flower.

FLOWERING OF SECOND-YEAR PLANTS AFTER CLIPPING, 1943.—Although the data for these series (table 2, 1943) are not based on as many plants as in the 1942 series, and some strains failed to flower on any treatment, in general the same conclusions may be derived. The practical failure of flowering of the nine northern strains on the 9-hour photoperiod, and the failure or great delay of the three southern strains in the 16-hour and N series, again suggest a fundamental genetic difference between the two groups of strains. The northern strains showed an increased delay in flowering on 16-hour and N photoperiods with decrease in latitude of origin.

FLOWERING SEASONS

The previous discussion is based primarily on the length of time required for the first plant to flower in each strain on each treatment. More significant conclusions are based on the sequence and duration of flowering of all plants. From data on the percentages of plants of each population which either had flowered or were in flower, and from the numbers of inflorescences which had appeared on individual plants at various times throughout both summers, graphs and a table were constructed (figs. 7, 8; table 4) depicting the flowering season of each strain on each treatment. These show the periods during which additional individual plants of each population first flowered (solid bars) and the continuing periods in which additional inflorescences appeared on one or more plants already in flower (broken bars). The graphs are best interpreted in conjunction with table 3, which shows the percentage of plants in each population which had flowered by the end of the season. Thus one can properly weigh the significance in figure 7 of the flowering season of strain 11 in the 9-hour series in 1942 against the 2% of the population represented (one individual), as compared with the flowering of the same strain on 16-, 20-hour, and N photoperiods represented by 16, 12, and 40% of the respective populations.

Table 3 indicates the great variation among strains as to percentages of the populations which flowered and also shows that some of these percentages were very low. Examination of the table as to trends in the percentages, both vertically and horizontally, and attention to those strains and treatments which failed to flower, suggest that a distinction can probably be drawn between percentages which were low because of the crowded condition of the plants and those which were low chiefly because of the inhibiting effect of a certain photoperiod on the flowering of the different strains. In this connection, those populations which began to flower earliest (table 2), when the plants were small, such as strain 1 in the 9- and 13-hour series and strain 12 on N, 16-, and 20-hour photoperiods, had the highest final percentages of plants in flower as compared with many of the other strains which were delayed in first flowering until the plants were much larger.

STRAIN 1.—Ten plants of strain 1 exserted inflorescences in three of the four series on natural photoperiod in the two summers (tables 3, 4; figs. 7, 8). These appeared between September 28 and October 12, when the effective light periods at Chicago (allowing for dawn and twilight) are between 12 and 13 hours, and were probably initiated not

more than a month or 6 weeks earlier under effective photoperiods not exceeding $13\frac{1}{2}$–14 hours. The ripe seed from which this strain was grown was harvested on September 15, 1941, at San Antonio, Texas, and the corresponding inflorescences may have been initiated on photoperiods of the same effective length in late July or early August as those at Chicago in late August or early September. That the delay in flowering until the short days of autumn at Chicago is actually the result of photoperiodic conditions is more conclusively indicated by the failure of all but three plants out of 140 to flower in the 16- and 20-hour series in the two seasons. These three plants were much delayed in flowering in comparison with those on 9- and 13-hour photoperiods (table 2), and their inflorescences were few in number and relatively sterile. The data thus show rather positively that many individuals of strain 1 have an upper critical photoperiod for flowering between 14 and 16 hours. This value may lie close to the maximum natural photoperiod to which they are subjected in their native environment in southern Texas. They are thus short-day plants, as are so many species of tropical and subtropical range (**22**). They flowered more abundantly and vigorously on a 13-hour than on a 9-hour photoperiod, however, although flowering first in the latter series in both years. Approximately two-thirds of the individuals in both 9-hour series (table 3) produced inflorescences during a period of 1 month (fig. 7). About half the flowering plants exserted more than one inflorescence each, the average number per flowering individual each year being 1.6. In both years flowering in the 9-hour series ceased around the end of July, although the plants remained green and were so small (fig. 2) that crowding was not a factor. This was in contrast to the re-

TABLE 4

SEASON OF FLOWERING* ON CHICAGO NATURAL PHOTOPERIOD, AS SHOWN BY COMBINED DATA FROM FOUR SERIES IN 1942–43

Strain no.	Season in which inflorescences first appeared on individual plants	Last date on which inflorescences appeared on plants in flower if different from column 2	No. of flowering individuals represented
12	May 15†–August 14	September 11	49
11	June 23–August 18	.....	25
10	May 15†–August 18	.....	10
9	July 1–August 18	August 28	20
8	July 5–September 23	.....	11
7	June 24–September 1	September 11	17
6	July 22–October 9	.....	4
5	July 5–September 23	September 25	18
4	July 5–October 9	.....	18
3	August 21‡–October 15	.....	50
2	July 5§–August 14	.....	4
1	September 28–October 12	.....	10

* Exsertion of inflorescence from surrounding leaf sheath.

† Flowered before clipping on May 21, 1943.

‡ Dates based on experiments in addition to series of 1942–43; inflorescences appearing in August were weak.

§ Inflorescences weak and relatively sterile.

sponses on the 13-hour photoperiod, where nearly all plants eventually flowered (table 3) and additional inflorescences were formed more or less continuously throughout both seasons (fig. 7) by relatively large plants (fig. 3). The average numbers of inflorescences per flowering plant in strain 1 in the 13-hour series were 3.3 and 4.4, respectively, in 1942 and 1943.

STRAIN 2.—Although originating within a few miles of strain 1, strain 2 did not respond in an identical manner. It resembled strain 3 more closely in many aspects of vegetative habit on all photoperiods (figs. 9, 10). It failed to flower abundantly on any treatment, possibly because of crowding. The nature of its photoperiodic responses as to flowering are probably best shown by the data for 1942 (tables 2, 3), seven out of twenty individuals flowering in the 13-hour series between 81 and 102 days after germination, while there was no flowering on other photoperiods during the season. In 1943, however, flowering occurred in only four individuals, all on natural photoperiod. These four plants, which had not flowered in 1942, exserted eleven weak and relatively sterile inflorescences between July 5 and August 14. Seven of them appeared in the last 2 weeks of July. If these inflorescences were initiated during the long days of June and early July, these individuals are not short-day plants so far as the production of visible inflorescences is concerned. The sterile condition, however, suggests the possibility that they may have been initiated during the shorter days of the spring months and developed only slowly to the stage of exsertion in the longer days of midsummer. These and other clones of this strain are now being investigated under other conditions. On the basis of the 1942 data, and from the nature of their vegetative responses, it is the writer's opinion that probably most of the individuals in this strain are intermediate or short-day plants.

STRAIN 3.—Except for less adverse effects of a 9-hour photoperiod on its growth, the responses of strain 3 from southern Arizona were somewhat similar to those of strain 1. Because of its vigorous vegetative growth on all photoperiods, the flowering of this strain was more adversely affected by crowding than were the other strains. While it did not flower in the 9-hour series in 1942, it did so in 1943. In other experiments in 1941 (**23**) sixty of a population of 100 plants of strain 3 flowered on an 8-hour photoperiod, although this percentage was smaller and the inflorescences less numerous and vigorous than for plants on a 12-hour photoperiod in 1941. The abundant data for the 3 years show that a photoperiod of 12–14 hours is more favorable for flowering of this strain than one of 8–9 hours, and that the upper critical photoperiod for flowering of most individuals lies between 14 and $14\frac{1}{2}$ hours (**23**), nearly the maximum to which it is naturally subjected. A very few plants flowered on a 16-hour photoperiod in 1941.

STRAINS 4–12.—Among the other nine strains, strain 12 from North Dakota flowered most abundantly, both as to individuals and in numbers of inflorescences. It produced inflorescences rapidly on 16- and 20-hour photoperiods in 1942 and 1943 (table 2), high percentages of the populations exserting them over a 2-month period in 1942 (table 3) and a lower percentage over a 1-month period in 1943. The average number of inflorescences per flowering plant ranged from 1.2 to 2.4 in the three series. In the four series on natural photoperiod in the

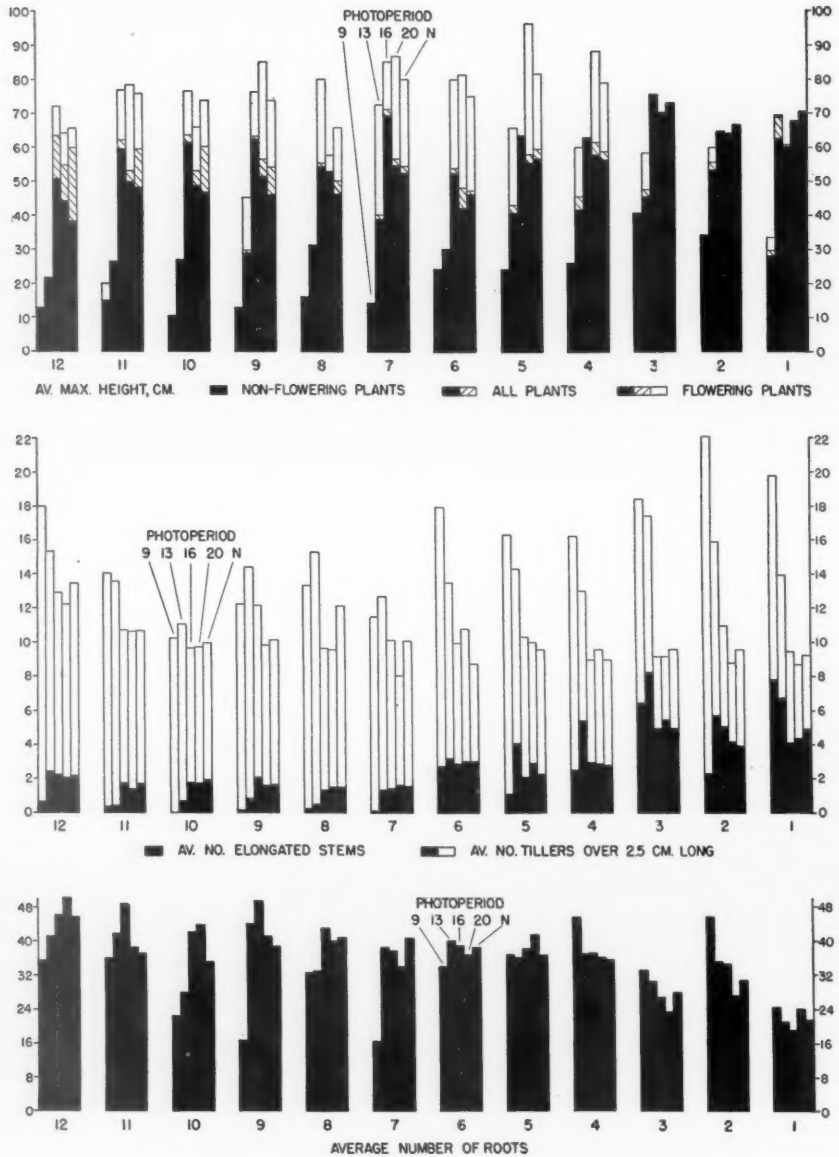


FIG. 9.—Average growth per plant of twelve strains of side-oats grama harvested in late September, 1942, after germination on April 11, 1942; grown on five photoperiods (hours) as indicated. Left to right (strains 12–1) the latitudinal sequence is north to south.

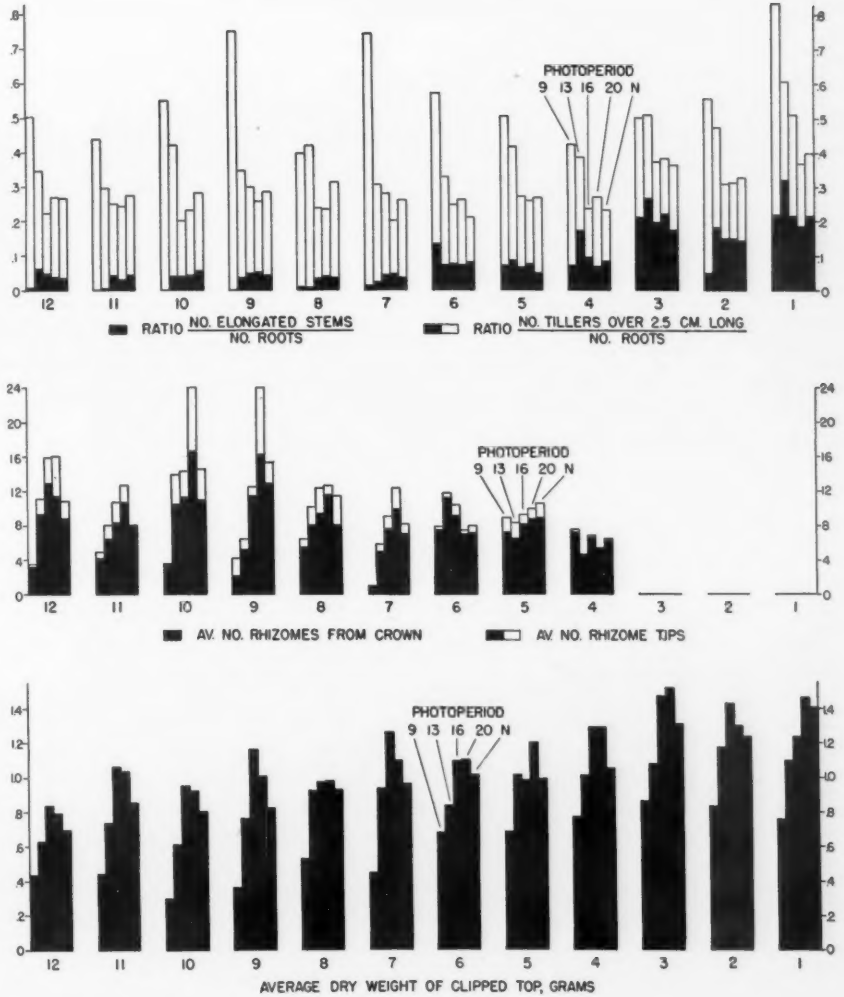

PHOTOPERIOD
9 13 16 20 N
12 11 10 9 8 7 6 5 4 3 2 1
RATIO NO. ELONGATED STEMS / NO. ROOTS
RATIO NO. TILLERS OVER 2.5 CM. LONG / NO. ROOTS
AV. NO. RHIZOMES FROM CROWN
AV. NO. RHIZOME TIPS
AVERAGE DRY WEIGHT OF CLIPPED TOP, GRAMS

FIG. 10.—Average growth per plant of twelve strains of side-oats grama harvested in late September, 1942, after germination on April 11, 1942; grown on five photoperiods (hours) as indicated. Left to right (strains 12–1) the latitudinal sequence is north to south.

2 years, inflorescences became visible between May 15 and September 11 (table 4), although the last plant to come into flower showed its first inflorescence on August 14, and it was the only plant exserting additional inflorescences after that date (fig. 8). Practically all these inflorescences must have been initiated between late April and the end of July, during which the effective natural photoperiods at Chicago exceed 14 hours. No flowering occurred in the 9- and 13-hour series in 1942, and only three plants flowered on these photoperiods in 1943. As mentioned earlier, the latter exserted only one sterile inflorescence each, which may well have been initiated in May on natural photoperiod and escaped clipping on May 21. These plants had exserted inflorescences in May before they were clipped. The flowering data thus indicate that strain 12 consists largely of long-day plants with a lower critical photoperiod of about 14 hours. This is well substantiated by the nature of the vegetative responses on shorter photoperiods.

The flowering seasons of the other eight strains (4–11) indicate that many of them may also consist largely of long-day plants, as shown by their failure to flower in the 9-hour series, while numerous individuals within these strains flowered on 16- and 20-hour photoperiods. Flowering in the 13-hour series, especially of the northern strains, was much more limited than on the longer light periods. The data for plants on natural photoperiod (see especially table 4 and the N-N series in fig. 8) suggest that the length of the lower critical photoperiod for flowering of the "late" plants of each strain decreases with decrease in latitude of origin in the nine northern strains. While apparently some plants in all these strains can initiate and mature inflorescences on the longest days of midsummer at Chicago (and on 16- and 20-hour photoperiods), the flowering season was progressively extended into late summer and autumn with decrease in latitude of origin of the various strains. Thus in strains 4, 5, and 6 some plants exserted their first inflorescences as late as the last week of September or early October. Some plants in these three strains also formed inflorescences on a 13-hour photoperiod. Thus, among the nine northern strains, the northern ones each seem to show more uniformity in photoperiodic response than the southern ones. It is even possible that strains 4, 5, and 6, for example, include both intermediate and long-day plants, although probably no short-day or day-neutral ones, since no plants of these strains flowered in the 9-hour series. Clonal divisions of "early" and "late" individuals of some strains are now being studied to determine more accurately their critical photoperiods and the exact status of the strains in photoperiodic classification. In general, it seems that many individuals in all twelve strains are probably able to flower most vigorously on photoperiods similar to those of the native environments in which they normally flower. The chief exception to this statement arises from the rather similar responses of some plants of the nine northern strains in the 16- and 20-hour series. Nowhere within its area of distribution is the species subjected to a natural photoperiod of 20 hours.

HOPKINS (19) grew nine strains at Hays, Kansas (approximately latitude 39°), under nursery conditions, all originating between the 33d and 47th parallels, and thus directly comparable only with strains 4–12 of the writer. In their second year, all of HOPKINS' strains be-

gan to flower in May and June, but apparently two of the southern strains showed most variation. His "Texas and Oklahoma plants (latitudes 35° and 36½°) started to flower on May 19th, but were not fully headed out until June 20th while the northern plants started on May 25th and were well in head by June 4th." He did not state how long flowering continued but reported that all plants were in full head on July 22. All his strains thus included individuals capable of maturing and probably of initiating inflorescences on photoperiods in excess of 14 hours, the effective photoperiod at Hays exceeding this value when the plants resumed growth in the spring. In their first year of growth, after planting on June 4, eight of his strains showed a more or less progressive lag in onset of first flowering (from July 17 to August 20) with decrease in latitude of origin (47° to 35°), comparable with the similar lag among the nine northern strains of the writer (fig. 7). His results thus confirm some of the conclusions of this study but shed no light on the most puzzling point—the differences between strains 1–3 and strains 4–12, since he had no material from the low latitudes of the first group.

ROGLER (**26**) reported that strains from Oklahoma, Texas, New Mexico, and Arizona (exact localities unspecified), grown in the nursery at Mandan, North Dakota (approximately latitude 47°), were so late in maturity that they were still vigorously growing at the time of the first autumn frosts, while northern strains had become dormant several weeks earlier. He gave no data on flowering seasons. Similar responses were reported by HOPKINS (**19**) at Hays, Kansas. The differing behavior of northern and southern strains may undoubtedly be attributed in part to the differing effects of the decreasing photoperiod upon internodal elongation, flowering, and other growth responses. In their native environments, the northern strains have become adjusted to a short growing season of long days, frost occurring while days are still fairly long, while the southern ones occur in areas with a long frost-free season, with no likelihood of frost until the short days of late October or November, or even later.

GROWTH IN HEIGHT

Maximum height per plant was measured periodically during the summer of 1942, and on all plants harvested in September, 1942, from the crown or cut stem ends to the tip of the longest outstretched leaf or inflorescence. The same trends in height growth soon became obvious in 1943, and no measurements were made. Final heights of all strains on all treatments when harvested in September, 1942, are shown by bar graphs of the averages for flowering, nonflowering, and all plants (fig. 9). The values for nonflowering plants were naturally considerably lower than for flowering plants in any one strain and treatment.

Height growth in all strains was least on the 9-hour photoperiod. In September, 1942, the six northern strains ranged from 10.5 to 16.2 cm. tall in this series, strains 4–6 from 24.0 to 26.1 cm., while strains 1–3 were much less adversely affected by the short photoperiod, strain 3 being the tallest (40.7 cm.). In the 13-hour series, except for strain 6, height was inversely correlated with latitude of origin, whether the average of all or only of nonflowering plants is considered, ranging from 21.8 cm. for strain 12 to 68.7 cm. for strain 1 (all plants). On 16-hour photoperiod, maximum heights of the different strains were more nearly

equal. The all-plant averages of eight strains (1, 2, 4, 5, 9, 10, 11, and 12) were between 60 and 65 cm. Strain 3 was tallest (75.7 cm.) and strain 6 shortest (53.7 cm.). In only strain 12 was the average based on nonflowering plants more than 2 or 3 cm. below that based on all plants. Height values on 20-hour photoperiod tended to be slightly lower than in the 16-hour series. Northern strains were relatively much taller in both 16- and 20-hour series compared with their heights on 9- and 13-hour photoperiods than were southern strains. In general, the range of other vegetative differences among these four treatments of one strain also increased with increase in latitude of origin of the strain (figs. 2–5). Height growth in the N series resembled that of the 16- and 20-hour series, but the final values were somewhat less in the six northern strains in the N series than on 16- and 20-hour light periods, probably because of the effect of the decreasing length of natural photoperiod in late summer on the activity of the apical meristem and on internodal elongation.

Periodic measurements through the summer showed that the nine northern strains reached their maximum heights in the 16-, 20-hour, and N series soon after August 1, or increased only slightly thereafter, whether flowering or nonflowering. Many vegetative culms on strains 1–3 in the same series, however, continued to elongate and form additional large leaves until they were harvested in late September. Strains 1–3 on the 9- and 13-hour series attained their maximum heights in the first 2 weeks of July, while in the more northern strains on these photoperiods height growth continued much later, although the latter plants were still very short when harvested. This contrasting behavior probably resulted in part from the short-day and long-day characteristics of the two groups of strains. In the long-day northern plants the longer photoperiods, initially most favorable for culm elongation and rapid growth in height, soon result in reproductive activity and a cessation of further height growth. In nonflowering plants or culms of these strains on long photoperiods there also seems to be a cessation of activity by the apical meristems when the culms reach a certain length, although the meristems apparently remain alive. In contrast, on the short photoperiods unfavorable for flowering in the northern strains many apical meristems remain active through the season, laying down new leaves rapidly and continuously, even though practically no internodal elongation occurs and the leaves are small when mature (figs. 2, 3). Rosettes are thus formed, but there is also a continuous though limited growth in height. More than twenty-five leaves were formed on many of the primary axes in strain 12 in the 9- and 13-hour series, although the average length of these shoots (to tip of longest leaf) was only 5.2 and 7.1 cm., respectively. This was in contrast to an average length of 63.1 cm. in the 16-hour series, the latter plants bearing approximately ten leaves per primary axis. In the short-day southern strains the short photoperiods favor early—although limited—internodal elongation, but the vegetative activity of the apical meristems is soon restricted. Some initiate inflorescences, and height growth ceases, while the final activity of others results in small leaves and short internodes. The long photoperiods allow maximum internodal elongation and the continued although not especially rapid formation of large leaves in strains 1, 2, and 3.

Behavior of primary axis

Tillers attained greater heights than the primary axis on nearly all plants of strains 4–12 in the 9- and 13-hour series and in strain 2 on 9-hour photoperiod. In strains 4–12, lengths of the primary axis were inversely correlated with latitude of origin in these series and were about one-half to one-third as long as the average tallest tiller per plant. In the 16-, 20-hour, and N series, the primary axes were equal to or taller than the tillers in most plants of all strains, as also in strains 1 and 3 in the 9- and 13-hour series and strain 2 in the 13-hour series. Nonflowering primary axes were alive throughout on nearly all plants in the 16-, 20-hour, and N series when harvested, but the upper portions of some had died on the shorter photoperiods, especially in the 13-hour series in all strains and in strains 1, 2, and 3 in the 9-hour series.

The effect of different photoperiods on the behavior of the primary axis is very striking, both among the strains and among the individuals of one strain. It is possible that correlations might be worked out which would make it possible to use the early behavior of the primary axis in the early selection of seedlings for "earliness" or "lateness," although no such attempt has been made in this study. Table 5 gives the percentages of plants in each strain and treatment which had produced one or more elongated internodes on the primary axis at the end of the first season. While internodal elongation is not necessarily correlated with reproductive activity in grasses, as is sometimes assumed, in some it is the first obvious sign of such initiation and may indicate conditions favorable for it. Nearly all plants of all twelve strains showed elongation in the N, 16-, and 20-hour series, as did strains 1–3 on the shorter light periods. In the six northern strains these final values on the 16-, 20-hour, and N photoperiods were attained at successively later dates in the summer with decrease in latitude of origin, even though some plants in all strains showed elongation by June 26. Final percentage values in these series were obtained for strains 1, 2, 3, and 12 by July 15, and for strains 4, 10, and 11 by July 29. The period of time over which individual plants began elongation was thus longer for the central than for the extreme northern or southern strains, suggesting the greater variability within the former, also indicated by the longer duration of their flowering seasons on natural photoperiods. The final percentages for the 13-hour series in table 5 also indicate such variability and suggest that the percentage of plants in each strain which have a critical photoperiod of less than 13 hours for elongation of the primary axis increases with decrease in latitude of origin.

The number of elongated internodes per primary axis, based only on plants possessing them, was inversely correlated with latitude of origin in the 13-hour series in strains 4–9 (5.02–1.33, respectively). The same measurement was positively correlated with length of photoperiod within all strains in the 9-, 13-, and 16-hour series. It is probable that the failure of internodal elongation in many plants of strains 4–12 on the short photoperiods was due to unfavorable photoperiod and not to other factors, such as crowding, which was of little significance in these series. The variability in internodal elongation thus shown within strains on the 13-hour series (table 5) is probably due mostly to genetic rather than to environmental causes. Strains 1, 2, and 3 were distinct in their

behavior, practically all plants showing internodal elongation on all treatments, although the final 100% values were attained earlier in the 9- and 13-hour series than in the others. It is obvious that internodal elongation of the primary axis does not indicate reproductive activity in these three strains.

TABLE 5

PERCENTAGES OF PLANTS WITH ELONGATED INTERNODES ON PRIMARY AXIS SEPTEMBER 23, 1942

STRAIN NO.	PHOTOPERIODS (HOURS)				
	9	13	16	20	N
12	0	0	100	100	98
11	0	0	94	96	98
10	0	0	100	90	100
9	0	4	100	86	94
8	0	10	90	100	90
7	0	16	88	86	88
6	35	80	100	95	100
5	14	86	94	96	100
4	16	86	96	94	100
3	100	100	100	100	100
2	95	100	100	100	100
1	100	100	100	100	100

TILLER DEVELOPMENT AND CULM ELONGATION

Total numbers of tillers and tiller buds over 2.5 cm. long were counted on plants harvested in late September, 1942 (fig. 9). In strains 7–10 the greatest numbers were formed on the 13-hour series, followed by the 9-hour series, while in strains 1–6 and 11 and 12 the values were inversely correlated with length of photoperiod in the 9-, 13-, and 16-hour series. In most strains, values for 20-hour and N series were near those of the 16-hour series. In all strains, basal branching was thus favored by the shorter photoperiods, most strikingly in strains 1, 2, and 3, somewhat less in 4, 5, and 6, and moderately in 7–12. Averages based on either flowering or nonflowering plants were very near those based on all plants, although in most strains and treatments the average for flowering plants was greatest.

On the shorter photoperiods, especially in the 9-hour series, numbers of tillers tended to be inversely correlated with latitude of origin, except in strains 11 and 12, which had higher values than some of the strains originating south of them. Differences in tiller number among strains on longer photoperiods were not statistically significant, except for the somewhat higher values in strains 11 and 12 in the 16- and 20-hour series. On natural photoperiod, tiller numbers in strains 8 and 12 were significantly greater than in the other strains, which showed little difference. With respect to different strains, these experiments thus show that only strain 12 may be considered to tiller relatively more profusely than any other strain on all treatments, in spite of the inverse correlation between latitude of origin and tiller number in the 9-hour series. In nursery-grown plants at Hays, Kansas, HOPKINS (19) reported greater numbers of tillers on southern than on northern strains, but he did not define a "tiller" as counted.

Tillers with one or more elongated internodes were counted in the plants harvested September, 1942. The surrounding leaf sheaths were stripped away to verify the elongation. All plants of strains 1, 2, and 3 in all series and nearly all plants of strains 4–12 on 16-, 20-hour, and N photoperiods had one or more tillers showing such elongation. In strains 4–12 in the 13-hour series, the percentages of plants showing elongation were 98, 92, 80, 52, 25, 36, 40, 22, and 66, respectively, while in the 9-hour series the values were 60, 22, 75, 8, 15, 10, 0, 12, and 26. Thus in the six northern

strains (except for strain 12, 13-hour series) less than half the plants on the two shorter photoperiods showed internodal elongation, and this was often found only in a limited number of tillers per plant showing slight elongation (a few millimeters) of one or more internodes. The inhibiting effect (or lack of stimulation) of the short photoperiods on internodal elongation and correlated limitations on foliage growth in the northern strains probably account in part for their growth being less vigorous than that of southern strains in nurseries in southern latitudes in the United States (**27**). They are there subjected to maximum photoperiods shorter than those optimum for their most vigorous growth.

The average numbers of elongated stems per plant, based on all plants, were not significantly different within strains 7–12 among the 16-, 20-hour, and N series, nor among these strains within any one of these series, except for the slightly higher values in strain 12 (fig. 9). The numbers of elongated stems were significantly higher in strains 4–6, and even more so in strains 1–3, on the long (16- and 20-hour) and N photoperiods than in more northern strains. Except in strains 1–3, the values for plants on the short photoperiods shown in figure 9 reveal no consistent trends, as they are based on all plants and are thus influenced by the different percentages of plants which showed elongation. Flowering plants consistently had greater numbers of elongated stems than had nonflowering plants in all strains and series.

The percentage of tillers showing elongation is a more useful comparative index among strains and treatments than the total numbers of elongated stems. In strains 1–3 on 16-, 20-hour, and N photoperiods, the former value was between 41 and 59%, in strains 4–6 between 20 and 35%, and in strains 7–12 between 12 and 20%. In the 13-hour series the percentages were 49, 36, 50, 41, 29, 24, 11, 3, 5, 6, 3, and 16 for strains 1–12, respectively; in the 9-hour series the sequence from south to north was 39, 11, 35, 15, 7, 15, 1, 2, 2, 0, 2, and 4%. Thus there is some tendency toward an inverse correlation between latitude of origin and percentage of tillers elongating in all the series, especially when the strains are grouped as 1–3, 4–6, and 7–12. This suggests that the northern strains are probably actually less vigorous in growth than the southern ones under many types of environment. Northern strains have been reported to be less vigorous than southern ones in several nurseries in different latitudes (**13, 19, 26, 27**).

Numbers of roots and rhizomes

One pot of each strain from each series was washed out in September, 1942, and the numbers of crown roots and rhizomes counted (figs. 9, 10). While the sample was probably inadequate, certain tendencies are suggested. Strains 1–4 had their highest numbers of roots in the 9-hour series, while among strains 6–12 this series had the lowest numbers. In strain 5 differences among series were slight. Numbers of roots in the four southern strains tended to be inversely correlated with length of photoperiod, while in the seven northern strains the correlation was direct. In all except the 9-hour series, the three southern strains (especially strain 1) had smaller numbers of roots than most of the other strains on comparable treatment.

The ratio of number of tillers over 2.5 cm. long/number of roots was highest

on the two short photoperiods in all strains and tended to be inversely correlated with length of photoperiod in the 9-hour, 13-hour, and 16-hour series. The ratio of number of elongated stems/number of roots was greatest in strains 1-3 because of the greater number of elongated stems and the smaller number of roots in those strains.

Rhizomes were developed on the nine northern strains only (fig. 10). Their absence on strains 1-3 again suggests that these three strains form a group apart from the others. In strains 4-12 rhizomes developed on practically all plants in the first summer, and when harvested in September some of them, in all except the 9-hour series, had sent up green shoots with leaves up to 12 inches long. The horizontal rhizomes tended to branch at varying distances from the crown, most markedly in strains 7-12 (fig. 10). In these strains the numbers of rhizomes from the crown tended to be directly correlated with length of photoperiod, while relationship to photoperiod was not obvious in strains 4-6. In strains 7-12 rhizome development was thus favored by the longer photoperiods, while tiller formation was favored by the shorter light periods, so that the ratio of tillers/rhizomes was inversely correlated with length of photoperiod. This latter relationship was also true in strains 5 and 6.

Top weight

Dry weights of tops as clipped in September, 1942, were determined for all plants (fig. 10). There were positive correlations between dry weight and length of photoperiod for all strains in the 9-, 13-, and 16-hour series. Weights of plants on the 20-hour treatment were near those on 16-hour. Southern strains weighed more than did the northern ones within all series, suggesting again that the former are inherently more vigorous. There was a tendency toward an inverse correlation between weight and latitude of origin within any one series. Similar results have been indicated for nursery-grown plants on natural photoperiods (27). Hopkins (19), at Hays, Kansas, reported that at the end of the first year of growth southern plants had produced approximately twice as much air-dry forage as had those from central latitudes and four times as much as had those from the north. A greater proportion of the weight was contributed by stem tissue in the southern strains than in the northern. This was also true in the experiments here reported, so that differences in weight of palatable forage would have been somewhat less than differences in total weight.

Discussion

This study has confirmed the reports of others that plants of side-oats grama from different latitudes are morphologically and physiologically distinct when grown together under similar experimental conditions. This diversity must therefore have a genetic basis. Each strain in turn shows considerable genetic differentiation. The study has further shown that this diversity involves adaptation to different photoperiods by the various strains. Plants of three strains from southern Texas and southern Arizona, in photoperiodic experiments, show most nearly normal (nearest to that under field conditions) vegetative and flowering behavior on a photoperiod (13 hours) close to those of their native growing season. Most of them fail to flower, although they grow luxuriantly, on photoperiods longer than 14 hours. They are not naturally subjected to such long photoperiods. Under nursery conditions

in more northern latitudes most of the individuals in these strains are thus delayed in flowering until the shorter days of autumn, when they are likely to be injured by frost, the latter often occurring while they are still in a vigorous vegetative condition. Under the conditions of this experiment these strains were also non-rhizomatous. In North Dakota and Kansas, southern strains are also prone to winter injury (**19, 26**). It it obvious, therefore, that they are not genetically favorable for acclimatization in latitudes very far north of their native environments, unless their vigorous erect vegetative habit would make it desirable to attempt selection of cold-resistant individuals capable of flowering on long photoperiods. Strains 1 and 3 include a very low percentage of plants able to flower on a 16-hour photoperiod. Selections desirable for use as hay could be made from these three strains.

A North Dakota strain, in contrast, consists of individuals showing normal (nearest to that under field conditions) vegetative and flowering behavior only on photoperiods of 14 hours or longer (long-day plants). In their natural growing season they are not subjected to photoperiods of much lower value. When grown on shorter photoperiods, most of them do not flower or show much internodal elongation, although they tiller abundantly to produce rosettes. Rhizomes are formed on the shorter photoperiods, but not so abundantly as on longer ones, and dry weight yields are also lower. When grown in southern latitudes in the United States, where maximum photoperiods do not exceed 14 hours for more than a few weeks, such a strain could not compare favorably with those from more southerly origins, irrespective of the suitability of other environmental conditions.

Strains from central latitudes in the United States, especially those from Oklahoma, show considerable variation in photoperiodic response within strains, although these responses tend to exhibit a gradient among strains correlated with latitude. Strains from Nebraska and Kansas apparently consist mostly of long-day plants but probably with shorter critical photoperiods than those from North Dakota. Strains from Oklahoma and New Mexico certainly include some long-day individuals and probably some which are intermediate in their photoperiodic requirements; that is, which flower only on an intermediate range of photoperiods, probably of lengths corresponding with the range to which they are naturally subjected in their growing seasons. Vegetative growth of these strains of central latitudinal origin is less adversely affected by short photoperiods with decrease in latitude of origin, while all exhibit luxuriant vegetative growth on long photoperiods. The strains from Oklahoma and New Mexico, because of their apparently greater genetic diversity, and behavior resembling that in the field over a wide range of photoperiodic conditions, would probably be best fitted—from the standpoint of photoperiodic requirements—for acclimatization to latitudes north or south of their own if they showed other characteristics making such introduction desirable. Within their own latitudes they show most promise for the selection of "early" and "late" individuals, so far as this behavior is correlated with photoperiod. The use of artificial photoperiodic control in such selection would be of value in breeding programs.

The different strains of side-oats grama in these experiments have exhibited practically the entire range of reported photoperiodic responses, and

as a species it cannot be classified photoperiodically except with reference to strains. This is striking evidence that it is unwise to attempt such classification when only a few plants have been observed, although many species have been listed as long-day, short-day, or day-neutral plants on the basis of the responses of one or a few clones. Such generalizations at the species level can be safely made only when strains from a wide latitudinal range have been investigated.

These experiments also show that the habit of a strain in one environment is not predictable from its mode of growth in another, as has been conclusively demonstrated by TURESSON (**32, 33, 34**) and CLAUSEN, KECK, and HIESEY (**7**). These investigators showed that genotypic differences not apparent in one environment were often revealed in another. The range of vegetative expression induced in one strain by the different photoperiods was greater in the northern than in the southern strains, and strain differences of a vegetative nature were accentuated by the shorter photoperiods. This is in contrast with the results of WERNER (**36**), who found that a simulated "northern" environment (longer photoperiods as one factor) gave opportunity for manifestation of more varietal differences among several varieties of potatoes than did a "southern" one. It also contrasts with the observations of CLAUSEN *et al.* (**7**), who found in their reciprocal transplant experiments that "ecotypical differences may be accentuated at one station over another, and the most vivid impressions of these differences are obtained at that garden where the species [*Potentilla glandulosa*] as a whole does its best." The first but not the latter part of their statement applies to the growth of side-oats grama in these experiments.

The wide range of morphological expression of the different strains induced by the various treatments casts some doubt on the general validity of the five biotypes of side-oats grama described by FULTS (**13**), based on plants from different latitudinal sources grown in one nursery of central latitude. It is problematical whether his descriptions would apply accurately to the same plants grown in nurseries in the northern or southern United States, where natural photoperiods of different length might accentuate or diminish the differences between individuals assigned to one or another of his biotypes.

There is little doubt that side-oats grama originated in low latitudes, possibly in southwestern United States or Mexico, and has spread north and south to higher latitudes in Argentina and southern Canada. The center of distribution of the genus is in Mexico and southwestern United States (**16**). Other species in the same subgenus (*Atheropogon*) are confined to low latitudes (under 35°), and plants in the genus with low chromosome numbers have been reported chiefly from this region (**13**). Most of the investigated individuals of side-oats grama are hexaploids ($2n = 42$), but FULTS (**13**) also reported chromosome numbers of 28, 35, 40, 45, 56, 70, and 98 in the species. The individual with $2n = 28$ was from Kansas, while the few plants with numbers higher than $2n = 42$ were from Oklahoma, Texas, Arizona, and Colorado. FULTS stated that "the production of new types through various chromosomal aberrations is probably still a dynamic process in this region," and that "it is likely that the diploid type of $2n = 14$ will be found by further

search, especially in the southwestern biotypes." FULTS' first statement may help explain in part the genetic variability of the writer's material from Oklahoma and New Mexico, although no chromosome counts have been made upon it. His data thus suggest, although they do not confirm, an origin and continuing evolution of side-oats grama in low latitudes, but apparently the hexaploid complex has been most involved in the spread to higher latitudes in the United States.

The present photoperiodic differentiation of the species is thus of interest in connection with its probable origin and evolution. The typical short-day response of the three southern strains could represent the original adaptation to the short photoperiods of low latitudes in which the species originated and in which these strains persist. Within these strains, however, a few individuals (possibly day-neutral, although clonal studies on different photoperiods are not completed) are able to flower on longer photoperiods. While of little importance in adjustment of these strains to their present environments, they suggest that races with similar ability, however it originated, would have been perpetuated through natural selection in a migration to higher latitudes in which flowering must necessarily occur in the long days of the frost-free season. Even with the development of the rhizome habit, it is probable that plants capable of flowering only on short photoperiods would have been eliminated or very restricted in their rate of migration if they persisted through vegetative propagation. The populations spreading north thus would have come to consist largely of day-neutral plants, or of intermediate or short-day plants with a very long critical photoperiod. Some of the "late" plants from Oklahoma and New Mexico may be of the latter type, but it is doubtful whether many individuals in these strains are day-neutral. The mode of evolution of the typical long-day response of the more northern strains is obscure. The ability to flower on a 20-hour photoperiod is not of survival value at present, and it is doubtful whether the species has ever ranged to latitudes where such photoperiods are encountered. Any complete evolutionary history of the species must also account for a loss of ability to flower on short photoperiods by the northern strains and their tendency to limited growth and dormancy as photoperiods decrease below a certain level. But whatever the mechanism of the evolutionary process back of such diversity, it has certainly resulted in a high degree of adjustment by the respective strains to the range of photoperiods encountered in their native environments during the season of the year otherwise favorable for growth. The ability of some individuals in most of the strains to grow and flower well over a wider range of photoperiods, while of no importance in their native environments, has undoubtedly been of evolutionary significance and of survival value in the migration of the species to different latitudes. Such variants hold most promise for acclimatization to areas north or south of their native ones and possibly for the ultimate segregation of desirable day-neutral types.

Summary

1. Twelve strains of side-oats grama (*Bouteloua curtipendula*), originating over a latitudinal range of approximately 17° from San Antonio, Texas, to Cannonball, North Dakota, have been grown

for a 2-year period in the greenhouses at the University of Chicago. Their responses on Chicago natural daylength and on 9-, 13-, 16-, and 20-hour photoperiods have been analyzed.

2. Vegetative and flowering responses on the different photoperiods showed that three strains from southern Texas and southern Arizona consist almost entirely of intermediate or short-day plants, with an upper critical photoperiod for flowering between 14 and 16 hours. These strains flowered more vigorously on a 13-hour than on a 9-hour photoperiod. A North Dakota strain consists largely of long-day plants with a critical photoperiod of about 14 hours. The other eight strains, from Nebraska, Oklahoma, Kansas, and New Mexico, also include numerous long-day individuals, although the data suggest that the length of the critical photoperiod for flowering of the "late" plants decreases with decrease in latitude of origin of these strains. The Oklahoma and New Mexico strains accordingly show more diversity in response within each strain on each treatment and may possibly include both intermediate and long-day plants. Many individuals in all twelve strains are probably able to flower most vigorously on photoperiods similar to those of their native environments on which they normally flower. Other responses indicate a similar nice adjustment to the range of photoperiods in the growing seasons of their native environments, although a few individuals in most strains grow and flower well over a wider range.

3. Internodal elongation and height growth were increasingly suppressed on a 9-hour photoperiod—and to a lesser extent on 13-hour photoperiod—with increase in latitude of origin. In the other series, maximum heights were more nearly equal, and other vegetative differences among strains were also less apparent. The development of the primary axis was more strongly correlated with strain origin and treatment than was that of the tillers, and its early behavior might be a useful criterion in genetic analyses. Tillers in all strains were more abundant on the shorter photoperiods than in the other series, but their numbers were not strikingly correlated with latitude of strain origin. The percentages of tillers showing internodal elongation tended to be correlated inversely with latitude of origin in all series. Numbers of crown roots tended to be correlated inversely with length of photoperiod in the four southern strains, while in seven northern strains the correlation was direct. In all except the 9-hour series, the three southern strains had smaller numbers of roots than most of the other strains on comparable treatment. Rhizomes were not produced by the three southern strains. Dry weights of clipped tops were directly correlated with length of photoperiod in any one strain and more or less inversely with latitude of origin among strains.

4. These results show definitely that a widely ranging native species may be so differentiated in its photoperiodic requirements that its assignment to a particular photoperiodic class may be generally invalid unless based on material from a wide latitudinal range. The results, and the conclusions based on them, also help to explain some of the differences which have been observed among similar latitudinal strains grown together in various nurseries. They also indicate the nature of the restriction which the adaptation of strains of this species to particular ranges of photo-

period imposes upon plant breeders, who might be aided by the use of artificial photoperiodic control in their programs. The possible origin of such photoperiodic differentiation should be of considerable interest to students of evolution and plant geography, since the species probably originated in low latitudes with short days and has therefore become secondarily adapted to the long days of higher latitudes.

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UNIVERSITY OF CHICAGO

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FUNGISTATIC ACTION OF DIPHENYL ON CITRUS FRUIT PATHOGENS[1]

G. B. RAMSEY,[2] M. A. SMITH,[3] AND B. C. HEIBERG[4]

Introduction

TOMPKINS (4) was the first to point out the value of diphenyl in controlling green-mold rot of oranges when used as impregnating material for paper wraps. When treated wraps were used, he noted that not only was the number of rotting fruits reduced but also the spore formation accompanying rotting.

Following these studies, FARKAS (1) showed from the results of extensive shipping tests with oranges from Palestine to England that the percentage of green mold (*Penicillium digitatum*) was six or seven times higher with untreated wraps than when diphenyl-impregnated wrappers were used. His tests on the physiological effects of diphenyl also showed that if this material is taken up at all by the fruit, it remains in the peel, and the quantities concerned are so minute that they are entirely negligible as compared with the amounts that can be tolerated by man (2).

FARKAS and AMAN (3), studying the action of diphenyl vapor on various molds, found that a concentration of 0.08 mg. per liter of air stopped development of *P. digitatum*, *P. italicum*, and *Diplodia* sp. completely. However, the spores and older hyphae continued to grow when the diphenyl was removed.

During the last 3 years there has been considerable increase in the use of diphenyl-impregnated wrappers for oranges, lemons, and grapefruit by commercial firms in the United States. Although the shipping tests abroad and those made in this country have shown diphenyl to be

[1] Investigation conducted by the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U.S. Department of Agriculture, in co-operation with the Department of Botany at the University of Chicago.

[2] Senior Pathologist, [3] Associate Pathologist, [4] Scientific Aid; Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U.S. Department of Agriculture.

effective in the control of the green and blue molds (*P. digitatum* and *P. italicum*) of citrus fruit, the manner of action of this material and its effect on spore germination and growth of these fungi have not been investigated. This paper records the results of studies of the action of diphenyl vapor on spore germination and growth in pure culture, not only of the blue and green molds but also of other of the more important citrus-rot fungi.

From among the many fungi that affect citrus fruit during transit, storage, and marketing, the following were selected for study as being the most serious pathogens: *Alternaria citri* Ell. and Pierce, *Botrytis cinerea* Pers., *Colletotrichum gloeosporioides* Penz., *Diplodia natalensis* P. Evans, *Phomopsis citri* Fawc., *Penicillium digitatum* Sacc., *P. italicum* Wehmer, *Phytophthora citrophthora* (R. E. Sm. & E. H. Sm.) Leonian, *Sclerotinia sclerotiorum* (Lib.) Dby., and *Trichoderma viride* Fr.

Preliminary tests indicated that the vapor from 0.05 gm. of diphenyl was effective in inhibiting spore germination and mycelial growth of some of the citrus-rot fungi in plate cultures and their spores in water drops; however, 0.2 gm. diphenyl was used in each test in order to assure a strong concentration of vapor.

The first method of studying the effect of diphenyl vapor on the growth of these fungi consisted in making plantings of mycelium from pure cultures on potato dextrose agar (pH 6.8). Diphenyl crystals were placed in the center of the inoculated plates in one series; a second series similarly inoculated but to which no diphenyl was added served as controls. Two plantings of the organism to be tested were made on each plate about midway between the crystals and the edge of the plate. Since diphenyl is only very slightly soluble in water at ordinary temperature, it was found satisfactory to place the crystals directly on the agar; but in tests of each organism at least one set of cultures was made in which the crystals were placed in glass rings in the center of the plate to prevent the possibility of any surface spread or diffusion of the chemical. In tests with fungi that would make some growth at 35° F., the diphenyl vapor was effective in controlling the growth if it had any fungistatic action on the organism at higher temperatures (60°–80°). The data presented in table 1 were selected as typical of several series of temperature tests on the growth of the rot-producing fungi of citrus fruit at 40° and 70° F. The lower temperature approximates that commonly found in refrigerator cars during transit and the higher temperature approximates that found in most retail stores.

The diameters of colonies of the fungi grown on agar plates with and without diphenyl were measured at regular intervals. The growth characteristics of each organism are shown in figure 1. The degree of inhibition is shown by comparing the diameters of the colonies (table 1).

Two methods were used in studying the effect of diphenyl vapor on spore germination. In the first, suspensions of the various fungi were made by placing spores from vigorously growing, 5–10-days old cultures into tubes containing 50 cc. of sterile water and shaking vigorously to break up spore clumps. Two cubic centimeters of each suspension was transferred to another tube containing 20 cc. of sterile water. These tubes were thoroughly shaken to insure uniformity of spore distribution, after which drops of the final suspension were pipetted to cover slips which were inverted over Van

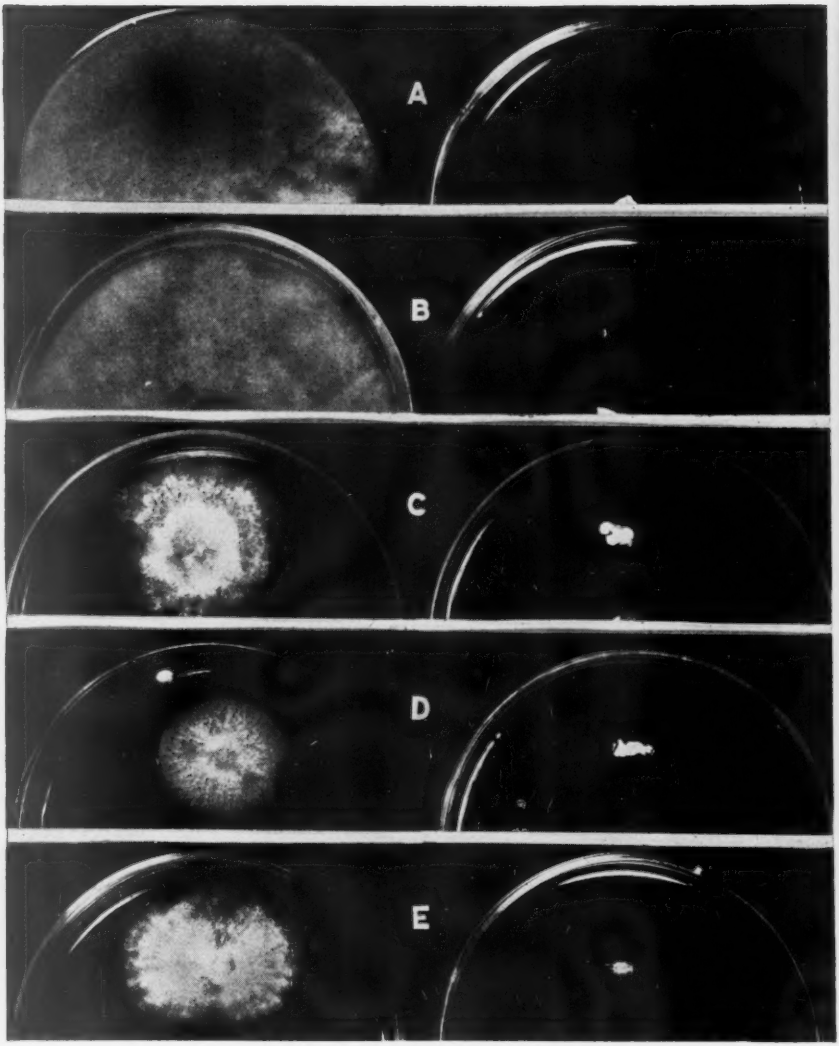

FIG. 1.—Effectiveness of diphenyl in controlling growth of citrus pathogens: left, control; right, treated. A, *Diplodia natalensis;* B, *Botrytis cinerea;* C, *Phomopsis citri;* D, *Penicillium italicum;* E, *P. digitatum;* F, *Sclerotinia sclerotiorum;* G, *Trichoderma viride;* H, *Colletotrichum gloeosporioides;* I, *Alternaria citri;* J, *Phytophthora citrophthora.* Cultures grown at 70°–75° F. and usually photographed the third day.

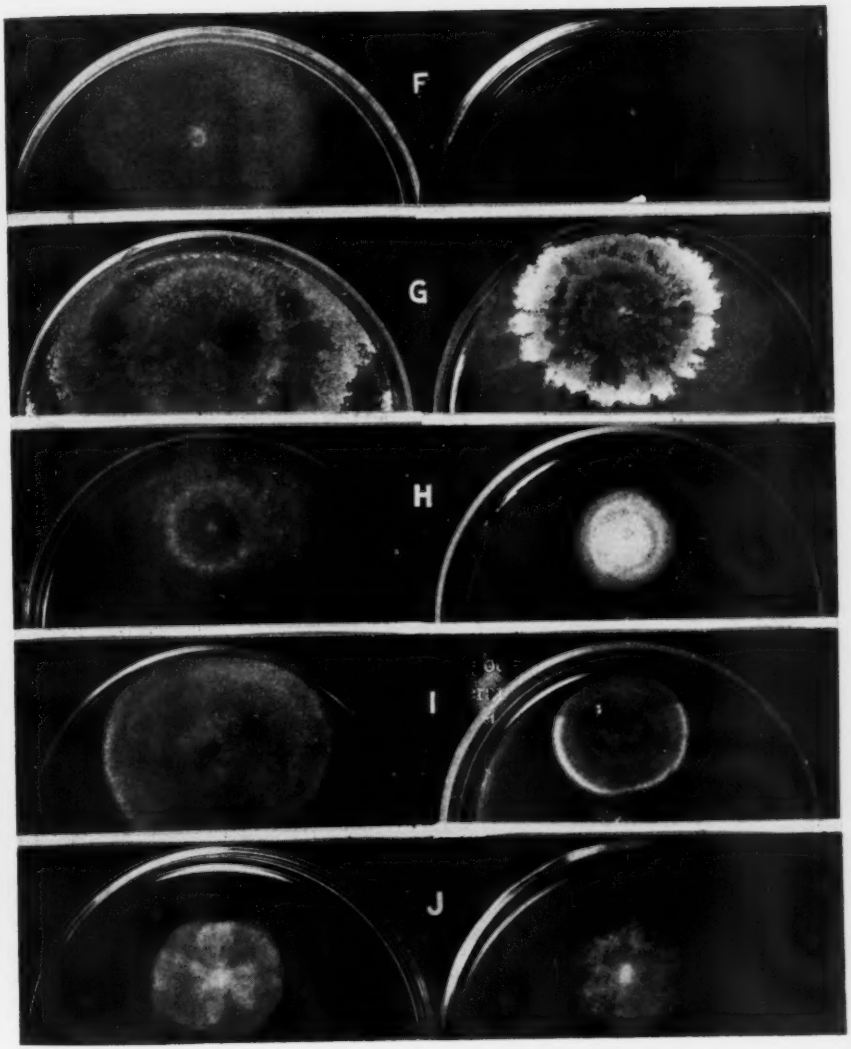

FIG. 1.—*Continued*

Tieghem cells affixed to glass slides. Diphenyl crystals (0.2 gm.) were placed in the bottom of these cells. Spore-containing drops inverted over Van Tieghem cells in which no diphenyl was added served as controls. Unless otherwise indicated, the cells were incubated at 75° F. All tests were made in duplicate. The percentage of germinated spores was determined by counts at the end of 24, 48, and 72 hours (table 2).

The second method consisted of the addition of two drops of the diluted spore suspension of the fungi to be studied to the surfaces of plates of potato dextrose agar by means of a sterile loop. The plates were then tilted to allow the drops to spread along the surface of the agar. In one series a small crystal of diphenyl was added to each of the plates. A second series, similarly inoculated but receiving no diphenyl, served as controls. Observations of the progress of spore germination were readily made by placing the plate on the stage of the microscope and making direct counts.

TABLE 1

GROWTH OF CITRUS FRUIT PATHOGENS ON AGAR PLATES

C, control; D, exposed to diphenyl vapor

PATHOGEN	TREATMENT	DIAMETER OF COLONIES (MM.)							
		Days at 40° F.				Days at 70° F.			
		5	7	9	21	1	3	5	7
Alternaria citri	C	4	8	11	25	8	35	57	76
	D	T*	2	4	18	5	20	33	46
Botrytis cinerea	C	5	14	31	80	7	54	80†	.....
	D	0	0	0	0	0	0	0	0
Colletotrichum gloeosporioides	C	0	0	0	0	T	32	56	74
	D	0	0	0	0	T	16	27	39
Diplodia natalensis	C	0	0	0	0	15	80	.....	.....
	D	0	0	0	0	0	0	0	0
Penicillium digitatum	C	0	0	0	4	T	23	46	63
	D	0	0	0	0	0	0	T	3
Penicillium italicum	C	T	3	4	17	4	28	59	80
	D	0	0	0	0	0	T	T	T
Phomopsis citri	C	0	0	0	3	0	20	40	57
	D	0	0	0	0	0	T	8	12
Phytophthora citrophthora	C	0	0	0	0	4	23	39	57
	D	0	0	0	0	T	27	57	80
Sclerotinia sclerotiorum	C	0	0	0	9	T	63	80	.....
	D	0	0	0	0	0	0	T	8
Trichoderma viride	C	0	0	0	T	7	68	80	.....
	D	0	0	0	0	0	17	61	80

* Trace of growth.

† Full plate.

Results

The reaction of the important citrus fruit pathogens to diphenyl vapor as shown by growth rate in plate culture and spore germination studies is shown in tables 1 and 2. It will be noted that a few fungi were totally inhibited in growth, some were checked at first and then grew moderately well later, while others were retarded only slightly. The only case of increased growth in the presence of diphenyl vapor was that found in cultures of *Phytophthora citrophthora*. In the many tests made with this fungus it always grew as fast or faster in diphenyl vapor than in the plates without this chemical. The colonies of this fungus produced in the control plates were white and appressed, with the margins sharp and regular, whereas in the diphenyl plates they were white and somewhat flocculent, with irregular margins (fig. 1*J*). A few apparently normal sporangia were produced within a week in the diphenyl vapor, but none were observed in the control plates under the same conditions.

Apparent recovery from the initial shock of the chemical was exhibited by *Sclerotinia* and *Trichoderma*. These fungi eventually grew all over the culture plates, even covering the diphenyl crystals (fig. 1*G*). Although the mycelium of *Trichoderma* was not quite normal, many spores were produced. The mycelium of *Sclerotinia* became cream-colored and more compact than normal. After several weeks' growth, a few yellowish sclerotia were observed in diphenyl-plate cultures. Apparently the ability to tolerate diphenyl vapor varies greatly with the species.

Regardless of whether growth was slightly or greatly inhibited, all the fungi showed one common characteristic reaction to the vapor. The newly developed hyphae were greater in diameter than normal and there was a great increase in the secondary branching. With the fungi that showed least growth, such as *Botrytis*, *Diplodia*, and *Penicillium* sp., there was a tendency to produce compact masses of hyphae made up of malformed or

TABLE 2

AVERAGE PERCENTAGE GERMINATION OF SPORES OF CITRUS FRUIT PATHOGENS IN WATER DROPS AND ON AGAR PLATES, EXPOSED TO DIPHENYL VAPOR AT 75° F.

Pathogen	Percentage germination after											
	24 hours				48 hours				72 hours			
	Control		Diphenyl		Control		Diphenyl		Control		Diphenyl	
	Water	Agar	Water	Agar	Water	Agar	Water	Agar	Water	Agar	Water	Agar
Alternaria citri	88	77	10	22	100	86	52	69	100	93	95	95
Botrytis cinerea	85	72	0	14	100	88	43	52	100	96	50	69
Colletotrichum gloeosporioides	81	69	30	52	83	74	85	79	83	89	85	88
Diplodia natalensis	85	98	3	6	100	99	7	11	100	99	12	16
Penicillium digitatum	10	47	6	12	50	56	13	18	62	77	17	24
Penicillium italicum	40	33	0	8	64	61	3	17	75	75	10	22
Phomopsis citri	90	79	9	20	95	84	16	39	95	88	19	46
Trichoderma viride	2	12	0	0	25	27	0	12	62	43	18	35

giant cells. White masses of hyphae bearing giant cells resembling spores were particularly conspicuous in *Penicillium* (fig. 2*A*). No normal green or blue spores were ever produced in the presence of diphenyl. On removal of the diphenyl from such cultures, however, the mycelium began to develop normally and the characteristic colored spores were formed within 2 or 3 days, showing that the chemical has a fungistatic rather than a fungicidal action. None of the fungi tested in pure culture have been killed by the vapors, even in concentrations many times stronger than necessary to inhibit growth.

The spore germination studies reflected about the same reactions to diphenyl vapor as were found in the agar-plate cultures. In a few instances it would appear that inhibition was somewhat less, in that a rather high percentage of spores germinated; but in such cases usually only short, thick, malformed germ tubes were produced and these often burst before they made much growth (fig. 2*C*). In the case of *Botrytis* and *Diplodia*, whose vegetative growth on agar plates is completely inhibited in the presence of diphenyl, the spores germinated moderately well but their germ tubes stopped growth when they reached a length equivalent to about two spore diameters (fig. 2*B*). Spores of *Diplodia* frequently became enlarged and distorted and then burst before germ tubes were developed, but *Botrytis* spores did not burst. The bursting of spores and germ tubes and the prolific branching of hyphae suggest that the diphenyl vapor

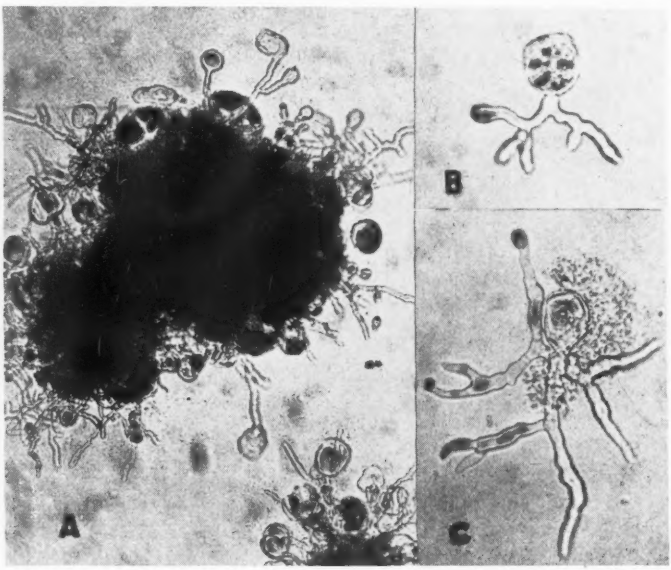

FIG. 2.—Characteristic growth of *Penicillium* and *Diplodia* in the presence of diphenyl vapor. *A*, giant cells and distorted hyphae of *P. digitatum*. Note lack of normal spores. Transmitted light. *B*, swollen spore of *D. natalensis* showing abnormal branching of germ tube. *C*, burst spore and contents of *D. natalensis* showing branching hyphae.

affects the plasma membrane in such a manner as to interfere with its regulatory powers, although death of cells does not occur except when they actually burst.

The control of rot of citrus fruit during transit, storage, and marketing by the use of diphenyl-treated wraps is evidently due to the inhibition of germination of contaminating surface spores, the retardation of growth of exposed hyphae already present on the fruit, and the prevention of new spore formation that often is the source of infection during handling.

Diphenyl-treated paper wraps are now successfully used for control of decay in many commercial shipments of citrus. During the course of this investigation another method of using the diphenyl as a fungistatic agent was demonstrated. Fiberboard boxes, such as are sometimes used for shipping apples, were fitted with paper-pulp trays containing twenty cuplike depressions for holding individual fruits (the Friday pack). Five trays were packed into each box. In one experiment 100 oranges from an apparently healthy lot of fruit were placed in trays impregnated with diphenyl and 100 were placed in untreated trays. The fruit in the treated trays was packed in one box and sealed, and the control fruit in untreated trays was packed in another box and sealed. At the end of a storage period of 3 weeks at 72° F. and relative humidity of 40%, the control box of fruit had 25% decay and the fruit in the treated trays had 1% decay. Figure 3 shows green mold rot in a treated and in an untreated tray. The vapors from the treated trays showed a fungistatic action on the rot-producing organisms such as was demonstrated in the pure culture studies.

Summary

1. The effect of diphenyl vapor on the growth of ten citrus fruit pathogens was studied. Growth of a few organisms was totally inhibited, some were moderately or only slightly checked, and one was apparently stimulated. Likewise, differences in response were observed when spores placed in water drops and on agar plates were exposed to diphenyl vapor. In the case of all the fungi so exposed, the developing hyphae were larger in diameter than normal and secondary branching was greatly increased. With some species of fungi there was a tendency to produce short, malformed or giant cells. In the presence of diphenyl the spores frequently became enlarged and distorted and often burst before germ tubes were developed to any appreciable extent. Death of cells did not occur except when they actually burst. Upon removal of diphenyl from cultures the organisms resumed growth and spore production, showing that the action of this chemical is fungistatic rather than fungicidal.

2. A new method of using diphenyl as a fungistatic agent in the control of citrus fruit decay was demonstrated. Unwrapped oranges placed in cuplike depressions in special paper-pulp trays impregnated with diphenyl developed little or no decay when packed in tight fiberboard boxes, whereas the controls developed considerable rot.

3. The control of rot in citrus fruit during transit, storage, and marketing through the use of diphenyl-treated wraps or trays is due to the inhibition of germination of contaminating surface spores, the retardation of growth of exposed hyphae already present on the

FIG. 3.—Effectiveness of diphenyl-treated trays in the control of green mold of oranges after 3 weeks at 72° F. and 40% relative humidity. *A*, diphenyl-treated tray; one decayed fruit out of 100 in test. Note white compact growth of mycelium and lack of spores. *B*, untreated tray, twenty-five decayed fruit out of 100 in test.

fruit, and the prevention of new spore formation that often is the source of infections during handling.

4. Diphenyl vapors should be effective in checking development of the following diseases of citrus fruit: blue mold rot (*Penicillium italicum*), *Botrytis* rot (*B. cinerea*), *Diplodia* stem-end rot (*D. natalensis*), green mold rot (*Penicillium digitatum*), and *Phomopsis* stem-end rot (*P. citri*). The failure of diphenyl to inhibit to any marked extent the mycelial growth and spore germination of the causal organisms of *Alternaria* rot (*A. citri*), anthracnose (*Colletotrichum gloeosporioides*), brown rot (*Phytopthora citrophthora*), cottony rot (*Sclerotinia sclerotiorium*), and *Trichoderma* rot (*T. viride*) suggests that these diseases are not likely to be controlled satisfactorily by this chemical.

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BELTSVILLE, MARYLAND

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EFFECT OF LIGHT INTENSITY AND NUTRIENT SUPPLY ON GROWTH AND PRODUCTION OF RUBBER AND SEEDS BY GUAYULE

J. W. MITCHELL,[1] A. GERALDINE WHITING,[2] AND H. M. BENEDICT[3]

Introduction

Environmental factors, such as temperature, moisture, soil condition, and light, vary widely throughout the geographic range of guayule in the United States, and as a result there are variations in the amount of rubber produced by the plants in the different localities.

MILOVIDOV (5) studied light intensity as a factor in rubber production by certain plants. The formation of plant mass by *Asclepias cornuti* was decreased by 19.25%, and that of krym-saghyz by 43.2%, as the result of subjecting the plants to light intensities that were 50% less than those which naturally prevailed. The amount of rubber produced was reduced to an even greater extent, indicating that light intensity was a relatively important factor in connection with rubber accumulation by these plants.

In comparing natural light conditions that prevail in different regions, it is necessary to rely on the relative number of hours of sunshine per year as an index rather than on actual intensity, since records are not available for the latter. It can be assumed, however, that the maximum intensities prevailing at relatively high elevations on a desert would exceed those recorded under more

[1] Physiologist, [2] Assistant Morphologist,[3] Associate Physiologist; Special Guayule Research Project, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U.S. Department of Agriculture.

humid conditions at lower elevations. With respect to light conditions that prevail at various locations in the guayule range, the average number of hours of sunshine per year at San Jose and Los Angeles (representing the central and southern coastal regions of California) was 6% less than that prevailing farther inland at Fresno, and 8% less than at El Paso, Texas (**7**)—the station nearest the natural guayule range at which hours of sunlight were recorded. On the basis of precipitation at Marathon and 02 Ranch, Texas, the percentage of sunshine that occurs on the natural guayule range in that state would appear to be even higher than at El Paso; but at Del Rio, Texas, where records were taken, 10% fewer hours of sunshine were recorded than for the coastal region of California.

Based on the percentage of total possible hours of sunlight, the yearly average at Beltsville, Maryland, where these experiments were undertaken, was lower by approximately 10–20% than that of most locations in the guayule range and approximately equal to that recorded at Del Rio. Furthermore, the relatively cold winters prevailing at Beltsville made it necessary to grow the plants in a greenhouse, where the light intensity was decreased still further by the glass during the period between October and February. Results presented therefore represent the behavior of plants grown in pots and subjected to a range of intensities, the mean of which was 10–30% less than that to which field plants are usually exposed. In the present experiments, plants grown in direct light at Beltsville attained, in a comparable period of time, a size equal to approximately one-third that of average irrigated field plants grown on the western guayule range and contained a slightly lower percentage of rubber (**3**).

Methods

Seedlings (variety 593) typical of those used for field plantings were obtained from the Alisal nursery of the Emergency Rubber Project near Salinas, California. They had grown from seeds during the previous summer season, and their branches had been trimmed to a length of 3–4 inches prior to transplanting. The seedlings were planted during the latter part of May in alluvial clay loam soil contained in 8-inch clay pots. They were then divided at random into eight groups, each containing ten uniform plants. Approximately 350 ml. of nutrient solution containing 35 milliequivalents of $Ca(NO_3)_2$ and 5 milliequivalents of KH_2PO_4 was applied to five pots of each group at intervals of from 3 to 6 weeks during the growing season, so as to maintain soil fertility. Soil in which the remaining plants grew was left untreated and decreased in fertility as the experiment progressed.

Two groups of ten plants each, including those growing in fertilized and also in unfertilized soil, were covered individually with frames over which was fastened a sufficient number of layers of tobacco cloth to reduce the light intensity to 75% of direct light, as measured by a Weston light meter. The intensity was reduced in a similar manner to 44% in the case of two other groups, to 24% of direct light in the case of two others, while the remaining two groups were left exposed to direct light. Treatments therefore consisted of four light intensities with two replications of each and two fertilizer treatments at each intensity level.

The frames used to support the cloth screen were approximately 3 feet wide, 6 feet long, and 2.5 feet high. The cloth screen with which these were covered extended to within approximately 6 inches

of ground level and slightly below the level of the rim of the pots, so as to allow for ventilation and to aid in equalizing the air temperature and humidity inside with that prevailing outside the tents. There was no appreciable difference in air temperature inside as compared with that outside the tents. Evaporation of water from the soil varied, depending on the light intensity. An attempt was made to maintain an optimum soil moisture content by the application of water to the soil when needed. No attempt was made to measure soil moisture content.

The seeds from the five plants of each replication of each treatment were collected during the growing season, which was divided into two parts—from June 1 to September 1 and from September 1 to October 1. After collecting, the seeds were cleansed and sorted into three sizes, with the aid of screens: those which passed a 10-mesh per inch screen, those which failed to pass through this but passed an 8-mesh screen, and those which failed to pass the 8-mesh screen. The seeds of each size gathered from the different treatments were then counted and subjected to germination tests.

In making these tests, four lots of 100 seeds were counted from each of the three samples. In cases where 400 seeds were not available, as many lots of 100 seeds as possible were obtained and the remainder used as one lot. Each lot of 100 seeds was treated and germinated separately. The 100 seeds were placed in shell vials and washed for 18 hours, three changes of 25 ml. of water being used in the washing. The seeds were then soaked for 2 hours in NaOCl (clorox) containing 1.5% available chlorine. Four milliliter of the hypochlorite was used for each lot of 100 seeds. The washing and soaking were carried out at room temperature. Following soaking in NaOCl, the seeds were rinsed several times, then placed while still moist between germination blotters in the germinators, and allowed to germinate at alternating temperatures of 20° C. for 17 hours and 30° C. for 7 hours daily. The tests were continued for 21 days, the germinated seeds being counted and removed from the blotters every 3 days. Repeated trials have shown that these treatment and germination conditions insure the germination of between 98% and 100% of the mature seeds.

Germination tests of seeds of various sizes are recorded as the means of two replications of 400 seeds each, or 800 seeds in all. The average percentage germination produced by the plants in each treatment was calculated from the total number and percentage germination of the seeds of each size. These values are given as the mean germination percentage of the seeds for each treatment.

The germination data were analyzed statistically according to the method for percentage outlined by HAYES and IMMER (2). The term significant is used in this paper to mean that there were odds of 19:1 against such differences occurring by chance.

The experiment was transferred to a greenhouse in the latter part of October where the day temperature was 60°–70° F. and the night temperature 35°–45° F.

On February 16, 1944, all plants were harvested. The plants were defoliated, the green functioning leaves removed separately from those that were brown but still attached, and finally the stems and roots were ground together into coarse pieces. After the fresh weights of these samples were recorded, they were dried in a well-ventilated oven at 65° C. and the dry weights determined. Rubber and resin determinations were made by

means of the SPENCE and CALDWELL method (6).

As related to growth and rubber production, attention was directed toward the effect of light intensities on the anatomy of leaves and stems, and particularly the effect on the structure and proportions of bark, since it is the major rubber-storing part of the plant.

Stem segments were taken at the time of harvest from each of three plants grown in each of the four levels of light and the two fertilizer treatments. A second replication was similarly sampled, thus giving a total of six plants studied per treatment. One piece, approximately 1 cm. in length, was cut from the base of the main stem. In most cases this piece was taken immediately above the area of the cotyledonary plate, but in others, where low branches were present, the sample was slightly higher. Identification of exact areas was possible externally by the crowded leaf scars and internally by the appearance of medullary resin canals, which, according to LLOYD (4), do not develop in the hypocotyl but within the lower crowded internodes of the true stem. Sections of the stem were cut at 40 μ with the aid of a sliding microtome equipped with a freezing attachment. The methods of preparing slides, obtaining measurements, and determining tissue areas have been described in an earlier paper (8). Cross-sectional areas of the pith, xylem, and bark, the last consisting of the tissues between the vascular cambium and cork cambium, were determined. Since the area of the bark functioning in rubber storage is reduced by the presence of fibers, cross-sectional areas of this tissue were also obtained. These data were subjected to an analysis of variance. In this paper the cross-sectional area of tissues was used as an index of their volume per unit length of stem.

Leaves for histological study were also collected from plants of each treatment. Sections were cut at 6 μ with the aid of a sliding microtome, stained with crystal violet, and mounted in glycerin jelly.

Results

GROWTH OF ROOTS, STEMS AND LEAVES.—A reduction of 25% in light intensity significantly reduced the dry weight of stems and roots of plants grown in fertile soil, and more extreme reductions in intensity were associated with successive reductions in stem growth (table 1; fig. 1). In contrast, the growth of plants in infertile soil was limited, and the weight of their stems was not influenced by even a 56% decrease in light intensity. However, an extreme reduction of 76% in intensity significantly reduced the combined weight of stems and roots. The fresh weight of the entire plant decreased steadily with reduction in light intensity in the case of those grown in fertile soil. In contrast, the growth of plants grown in infertile soil was favored by a moderate amount of shading, while full sunlight or an extremely low light intensity (24%) decreased the amount of growth as measured by fresh weight of the entire plant.

Full light intensity did not favor the production of leaves by plants grown in infertile soil, and this factor largely accounted for the reduction in the weight of plants grown at the maximum intensity as compared with that of others which were shaded lightly.

The ability of plants to retain their leaves during the winter months varied in proportion to the intensity of light to which the plants were exposed. In Feb-

ruary, approximately two-thirds of the total dry weight of the leaves of plants grown in the highest intensity was represented by those that were green and functioning, while only one-third of the leaves of plants grown in a reduced intensity (24% level) had remained green and were functioning at that time.

SEED PRODUCTION.—Considering first the plants supplied with additional nutrient, the greatest number of seeds was produced during June, July, and August by those plants grown under reduced light intensities (44%–75% of direct light). However, an extreme reduction in light intensity was unfavorable to the production of great numbers of seeds (table 2). During September, a greater number of seeds was produced by plants grown in direct light than by any others that were exposed to light of lower intensity. In the case of plants not supplied with additional nutrient, the greatest number of seeds was produced by those grown with a 56% reduction in light intensity during June, July, and August, and a 25% reduction during September.

The greatest proportion of large-sized seeds was produced by plants grown with added nutrient and shaded lightly (75% of full intensity). Marked reduction in numbers of large-sized seeds resulted when the light intensity was reduced to less than 56% of unfiltered light. Withholding nutrients tended to increase the proportion of small and decrease the proportion of large seeds.

The largest seeds gave the highest percentage of germination, the smallest seeds the lowest, and the medium-sized seeds an intermediate percentage. The germination of seeds gathered during September was significantly greater than that of seeds gathered during June, July, and August. While there were considerable differences in the number of seeds produced in the different treatments, there were no significant differences in the percentage germination (table 3).

ANATOMY.—Considering first the plants to which fertilizer was added, the stems showed a progressive decrease of the total cross-sectional areas in the three lower light intensities (table 4; fig.

TABLE 1

WEIGHT OF LEAVES, ROOTS AND STEMS, AND ENTIRE POTTED PLANTS OF GUAYULE GROWN FOR 8 MONTHS UNDER DIFFERENT LIGHT INTENSITIES AND LEVELS OF NUTRITION. INTENSITY EXPRESSED AS PERCENTAGE OF DIRECT LIGHT. WEIGHTS REPRESENT AVERAGES OF TEN PLANTS EACH

PERCENTAGE INTENSITY	FRESH WT. WHOLE PLANT (GM.)		DRY WT. STEMS AND ROOTS (GM.)		DRY WT. TOTAL LEAVES (GM.)		TOTAL LEAVES AS PERCENTAGE OF WHOLE PLANT (DRY WT. BASIS)		GREEN LEAVES AS PERCENTAGE OF TOTAL LEAVES (DRY WT. BASIS)	
	Nutrient added									
	+	−	+	−	+	−	+	−	+	−
100	66.7	36.7	37.2	19.5	15.0	3.5	28.7	15.4	67.7	65.5
75	63.3	40.6	31.8	19.6	13.8	5.5	30.3	22.8	56.3	51.7
44	55.0	39.0	27.5	19.3	11.7	5.2	29.7	21.3	54.0	50.8
24	38.9	30.9	18.3	14.8	7.6	5.1	28.8	25.4	29.8	30.4

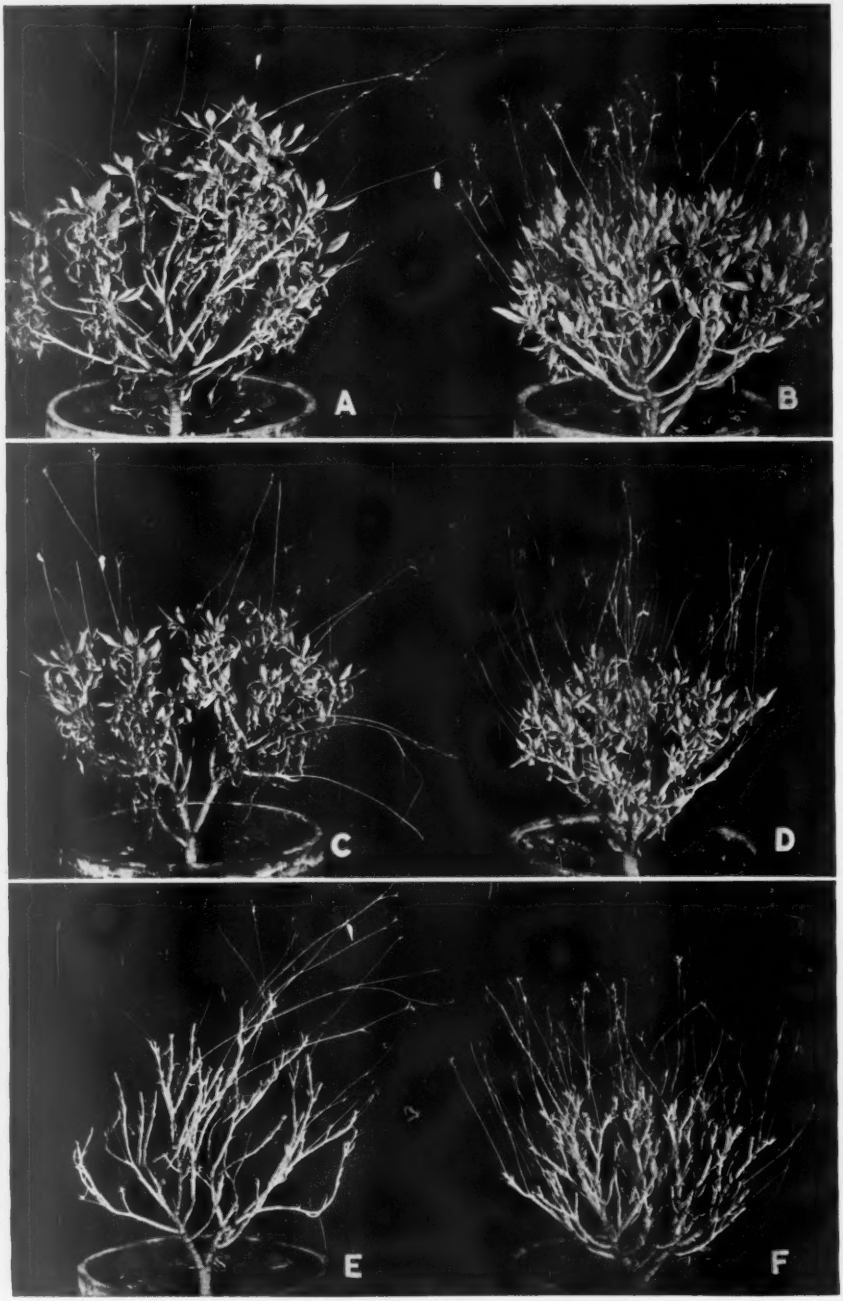

FIG. 1.—Guayule grown for 5 months during spring, summer, and fall under field conditions, followed by 4 months in greenhouse: *A*, in 24% of full light intensity and in soil to which nutrients were added; *B*, in soil to which nutrients were added and in full intensity; *C*, 24% of full intensity and without added nutrients; *D*, full intensity and without added nutrients; *E*, 24% of full intensity with nutrients added, plant after defoliation; *F*, full intensity and with nutrients added. Note effect of shading on number of branches, flower stalks, and dead leaves.

2). The stem area of those plants grown in direct light was not significantly different from that of other plants exposed to the 75% level. The areas of the different tissues composing the stem also decreased progressively in the three lower light intensities but did not differ significantly between the 75% level and direct light. There was a marked constancy of the proportions of each tissue, despite significant changes in over-all size of the stem at the different light intensities. The percentage area of bark showed no significant differences, although the actual area decreased by approximately 36%. Likewise, there were no significant differences in the percentage area of fiber in the bark of plants exposed to different light intensities, although the actual area of fiber was reduced by approximately 35%.

There was little difference in cell size or organization of parenchyma in the bark of plants grown with added nutrient and exposed to the different levels of light. Differences in extent of the tissue were apparently due to the number of cells produced in secondary growth, rather than to changes in the character of the cell. Large fiber cells in the bark were somewhat more numerous, and their walls somewhat thinner, in the shaded plants than in those exposed to unfiltered light.

In those plants grown without added nutrient, the actual cross-sectional area —both of the whole stem and of the various tissues—decreased significantly

TABLE 2

NUMBER OF SEEDS OF DIFFERENT SIZES PRODUCED BY GUAYULE PLANTS GROWN UNDER VARIOUS LIGHT INTENSITIES AND NUTRIENT CONDITIONS. INTENSITY EXPRESSED AS PERCENTAGE OF DIRECT LIGHT

Seed size	Collected June 1 to September 1, 1943				Collected September 1 to October 1, 1943			
	Percentage intensity							
	24	44	75	100	24	44	75	100
	Nutrient added							
Large	214	1484	1519	1376	89	259	370	295
Medium	1729	2179	3221	2003	506	1523	2024	3291
Small	327	272	321	437	94	274	259	290
Total	2270	3935	5061	3816	689	2056	2653	3876
	Nutrient not added							
Large	169	553	865	626	35	82	148	29
Medium	1707	2745	2000	1580	219	689	856	511
Small	220	252	250	261	45	111	132	299
Total	2096	3550	3115	2467	299	882	1136	839

TABLE 3

PERCENTAGE OF VIABLE SEED PRODUCED BY GUAYULE AT DIFFERENT SEASONS OF YEAR AND UNDER VARIOUS LIGHT INTENSITY AND NUTRIENT LEVELS. INTENSITY EXPRESSED AS PERCENTAGE OF THAT OF UNSCREENED LIGHT

Seed size	Collected June 1 to September 1				Collected September 1 to October 1			
	Percentage intensity							
	24	44	75	100	24	44	75	100
	Nutrient added							
Large	56.75	54.75	47.50	52.25	64.50	70.50	64.25	63.75
Medium	43.00	30.75	40.00	34.25	44.25	51.50	49.50	50.00
Small	13.75	9.25	7.50	10.50	15.50	21.00	19.00	29.50
Mean	39.42	39.66	41.00	38.82	42.75	49.75	48.68	49.26
	Nutrient not added							
Large	68.00	56.25	48.25	51.50	67.00	60.00	70.00	58.25
Medium	40.75	29.25	31.50	28.75	48.75	51.50	53.25	48.00
Small	15.25	13.50	10.50	7.00	30.75	21.75	24.00	24.25
Mean	40.09	32.21	34.36	31.99	47.30	48.84	51.80	40.55

TABLE 4

EFFECT OF DIFFERENT LEVELS OF LIGHT INTENSITY AND NUTRIENT ON AVERAGE CROSS-SECTIONAL AREAS OF VARIOUS STEM TISSUES AND ON AVERAGE PERCENTAGE OF CROSS-SECTIONAL AREA OCCUPIED BY EACH TISSUE. INTENSITY EXPRESSED AS PERCENTAGE OF DIRECT LIGHT. AREA VALUES REPRESENT AVERAGES OF SIX PLANTS EACH

Percentage intensity	Stem		Pith				Xylem				Bark				Fibers			
	Total area (mm^2)		Area (mm^2)		Total stem area (%)		Area (mm^2)		Total stem area (%)		Area (mm^2)		Total stem area (%)		Area (mm^2)		Bark area (%)	
	Nutrient added																	
	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
100	112.6	89.5	2.4	2.6	2.1	2.9	49.9	37.3	43.6	41.6	60.3	49.7	54.3	55.5	13.3	12.6	22.5	25.5
75	111.3	93.3	2.2	2.4	2.3	2.5	46.4	39.2	41.0	42.0	62.6	51.7	56.7	55.5	14.9	12.5	23.1	24.1
44	96.0	82.1	2.4	2.2	2.5	2.6	40.0	33.2	41.6	40.4	53.7	46.0	55.9	57.0	11.9	10.4	22.2	22.1
24	67.3	59.9	1.9	1.8	2.8	3.0	27.1	24.1	40.1	40.3	38.3	34.0	57.0	56.8	8.7	7.6	22.7	22.4

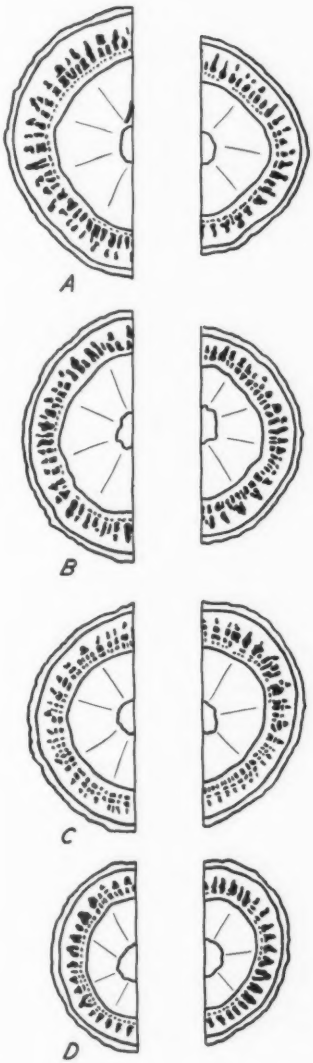

FIG. 2.—Tracings of projected images of stem cross-sections of guayule, those in left-hand column from plants grown in soil with nutrients added, right-hand column without added nutrients. *A*, plants grown in full light intensity; *B*, 75%; *C*, 44%; and *D*, 24% of full intensity. Areas of bark fiber darkened.

among the three lower levels of light, but there was no significant difference between the two highest levels. Greatest cross-sectional area of the component stem tissues occurred at the 75% light intensity level. The percentage areas of stem tissues of plants grown at the different light intensities and the different nutrient levels did not differ significantly.

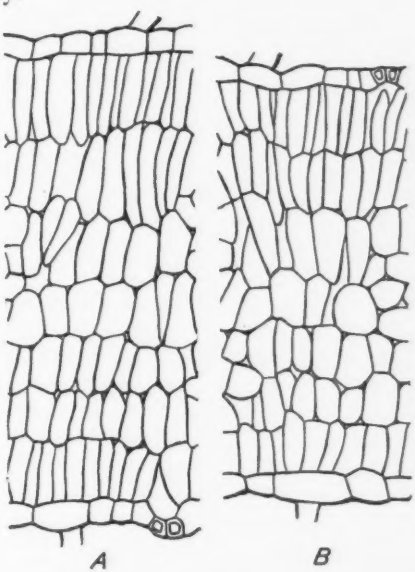

FIG. 3.—Cross-sections of leaves from guayule plants grown in soil to which nutrients were added: *A*, at full light intensity; *B*, at 24% of full intensity. Note only slight effect of shading.

Leaves of plants grown in the more fertile soil showed only a limited response to light intensity (fig. 3) with respect to amount and character of palisade and spongy parenchyma tissues. Leaf thickness decreased only slightly with shading. Leaves of plants grown at 75% of light intensity showed a 1% decrease in thickness compared with those of plants grown in direct light (6% and 12%, respectively, for plants exposed to 44% and 24% of full light intensity). GOUR-

LEY and NIGHTINGALE (1) found that in apple the leaves of plants in full light were 90% thicker than those of shaded plants, but in peach the sun leaves were only 20% thicker than shaded ones.

TABLE 5

PERCENTAGE AND ABSOLUTE AMOUNTS OF RUBBER IN STEMS AND ROOTS OF POTTED GUAYULE PLANTS GROWN FOR 9 MONTHS UNDER DIFFERENT LIGHT INTENSITIES AND NUTRIENT LEVELS. INTENSITY EXPRESSED AS PERCENTAGE OF DIRECT LIGHT. VALUES REPRESENT AVERAGES OF EIGHT PLANTS EACH

PERCENTAGE INTENSITY	RUBBER (%)		RUBBER PER PLANT (GM.)		POUNDS RUBBER PER ACRE (11000 PLANTS)	
	Nutrient added					
	+	−	+	−	+	−
100	5.13	3.71	1.91	0.69	46.4	16.8
75	3.88	3.38	1.23	0.66	29.9	16.0
44	3.43	3.39	0.94	0.64	22.8	15.5
24	1.92	2.60	0.34	0.37	8.3	9.0

Peach shed its leaves prematurely under shaded conditions. Likewise, the structure of guayule leaves was not changed appreciably by shading, and a marked increase in the proportion of dead leaves was noted in the case of heavily shaded plants.

RUBBER CONTENT.—Considering first the plants to which nutrient was added, a relatively high percentage of rubber accumulated in the stems of those grown in direct light (table 5). The stems of those grown in an intensity 25% less than that of direct light contained significantly less rubber on the absolute basis than did others grown in full light. More extreme shading of the plants was accompanied by a corresponding decrease in percentage stored. In contrast, both the percentage and the absolute amount of rubber in stems of plants grown without added nutrient were relatively low, and neither was significantly reduced by shading except at the lowest light level (24% of unscreened light).

RESIN CONTENT.—The percentage of resin in stems and roots of plants exposed to full light intensity was not influenced by the addition of nutrients (table 6), and the percentage in similar parts of shaded plants was greater in low nutrient plants than in those to which nutrients were added. Owing to their smaller size, however, the absolute amount of resin in plants grown without added nutrient was considerably less than that found in those grown in the more fertile soil. Moderate shading (75% of full intensity) resulted in a 25% decrease in the resin output of plants to which additional nutrients were supplied, in contrast to a 2% reduction in the resin output of those to which nutrients were not added.

TABLE 6

PERCENTAGE AND ABSOLUTE AMOUNTS OF RESIN IN STEMS AND ROOTS OF GUAYULE GROWN FOR 9 MONTHS UNDER DIFFERENT LIGHT INTENSITIES AND NUTRIENT LEVELS. INTENSITY EXPRESSED AS PERCENTAGE OF DIRECT LIGHT. RESIN VALUES REPRESENT AVERAGES OF TEN PLANTS EACH

PERCENTAGE INTENSITY	RESIN (%)		RESIN PER PLANT (GM.)	
	Nutrient added			
	+	−	+	−
100	5.0	5.0	1.86	0.98
75	4.4	4.9	1.40	0.96
44	4.3	4.6	1.18	0.89
24	3.2	3.6	0.59	0.53

Discussion

When grown at a relatively high nutrient level, plants of the present experiment were extremely sensitive to reduction in light intensity with respect to their total output of rubber. The most moderate shading reduced by 36% the absolute amount of rubber produced. There was an approximate linear relationship between the amount of rubber stored per plant and the intensity of light to which the plants were exposed (fig. 4). Even at the highest intensity used, light was a major limiting factor with respect to the total amount of rubber accumulated by plants grown in the more fertile soil. Shading was also associated with a decrease in concentration of rubber in the roots and stem tissues, and with a decrease in the over-all growth of the plants, as reflected in the weight of the leaves, roots and stem. Decrease in the stem diameter and cross-sectional areas of the various tissues occurred in the three lower light intensities, but there was no significant difference between the cross-sectional areas of those exposed to 75% and 100% of the intensity of direct light. The decrease in stem weight associated with moderate shading (75% level) may be accounted for on the basis of a decrease in the number of branches, an effect which became more striking as the light intensity was reduced, and which was most clearly evident in plants subjected to the lowest intensity (fig. 1*E*, *F*).

In contrast, those plants grown at a low nutrient level were relatively insensitive to variations in light intensity. Their vegetative growth and rubber content were significantly less than that of plants grown with added nutrient, irrespective of the intensity in which they were grown. Prolonged inhibition of vegetative growth, the result of maintaining the plants on a low nutrient level, was not associated with accumulation of carbon in the form of rubber, even in the case of plants exposed to direct light. Apparently the process of rubber synthesis, as well as the synthesis of other materials by these plants, was limited by an inadequate inorganic nutrient supply. Synthesis of resin, on the other hand, was not limited by an inadequate nutrient supply, since the percentage of resin in the stems and roots of the smaller low nutrient plants was equal to or greater than that recorded for the larger plants to which nutrients were supplied.

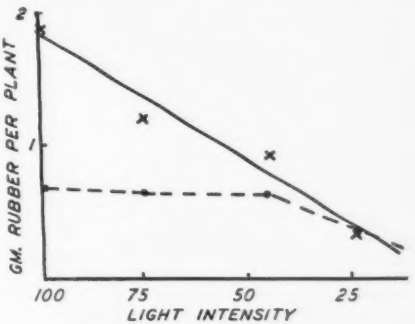

FIG. 4.—Effect of shading on amount of rubber produced by guayule grown at different nutrient levels and light intensities. Intensity expressed as percentage of that of unscreened light at Beltsville. Solid line represents plants to which additional nutrient was supplied; broken line, those grown without added nutrient.

Reduction in light intensity was associated with marked decrease in the percentage of leaves which remained green and functioning during the winter. The limited anatomical response of leaves to shading may have in part accounted for the fact that heavily shaded plants lost most of their green leaves during the winter season. This factor may have reduced to some extent the rubber-producing capacity of the shaded plants. The

tendency for moderately shaded plants grown in the more fertile soil to produce a relatively great number of seeds during the summer months may also have been a factor in limiting the amount of rubber produced by these plants during the following dormant season. The relative proportions of tissues composing the stem show no relationship to changes in nutrient treatment, variation in light intensity, or rubber concentration. The amount of rubber produced by shaded plants was therefore not limited by available rubber-storing area. It is evident that the limited rubber output of shaded plants grown with added nutrient was associated with (*a*) a decrease in the total synthesis of solid matter by the plant, (*b*) an increase in seed production under conditions of moderate shading, and (*c*) a decrease in the number of functional leaves during the winter rubber-storing season.

Summary

1. Potted plants of guayule were grown for a period of 5 months during the spring, summer, and fall in natural light of full intensity, and other plants were subjected to lower intensities obtained by means of cloth filters. Half the total number of plants were supplied with nutrient at intervals to maintain soil fertility, while the remainder were grown in relatively infertile soil. During the winter the experiment was transferred to a greenhouse and continued under controlled temperature conditions.

2. A 25% reduction in light intensity was associated with a 36% reduction in the amount of rubber produced by plants grown in relatively fertile soil. Further reduction of light was associated with a corresponding decrease in the percentage and absolute amount of rubber stored. The percentage and absolute amounts of rubber produced by plants grown in infertile soil were relatively low and not greatly affected by shading.

3. A reduction of 25% in light intensity reduced the dry weight of stems and roots of plants grown in fertile soil by 14.5%; and more extreme reduction in light further retarded vegetative growth. Of plants grown in infertile soil, the greatest leaf weight was produced by those exposed to 75% of full sunlight, but moderate shading had no appreciable effect on the weight of stem and roots.

4. Two-thirds of the leaves of plants grown in direct light remained green throughout the winter, but only one-third of those on deeply shaded plants remained green and functioning.

5. Reduced light intensity (44%–75% of unscreened light) favored seed production during June, July, and August. Shading was less effective in stimulating seed production during September. Decrease in light intensity was associated with decrease in the proportion of large seeds produced. Withholding inorganic nutrients was associated with an increase in the proportion of small and a decrease in the proportion of large seeds. Reduction in light intensity was not associated with an appreciable change in seed quality.

6. The cross-sectional area of stems was not significantly affected by a 25% reduction in intensity; but further reduction was associated with marked decrease in stem thickness, cross-sectional area of pith, xylem, bark, and fiber tissue in the bark.

7. Percentage areas of the various stem tissues were essentially constant both in the different light intensities and with the two fertilizer treatments. The percentage of bark was not correlated with changes in percentage of rubber.

8. Under full light intensity, the per-

centage of resin in plants grown with a limited nutrient supply was equal to that of plants to which nutrients were supplied; and with shading those subjected to a limited nutrient supply contained a higher percentage of resin than did those afforded nutrients.

All rubber and resin determinations were made by Mr. R. L. HOLMES and statistical analyses by Mrs. MARY W. SHANOR.

BUREAU OF PLANT INDUSTRY, SOILS AND AGRICULTURAL ENGINEERING
BELTSVILLE, MARYLAND

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WINTER HARDINESS IN GUAYULE

JOHN W. MITCHELL[1]

Introduction

Plants of guayule have withstood winter temperatures of 5° F. at Marathon, Texas, and 10° F. at Tuscon, Arizona (3). A temperature of −5° F. has been recorded at El Paso, Texas, near the geographic range of guayule. At Fort Stockton, Texas, within the natural range and near its northern limit, a minimum of −7° F. has been recorded. From this it appears that this plant is able to withstand relatively low temperatures, at least for a short time, in some localities in the United States.

On the other hand, Russian investigators have reported (2, 4) that the varieties with which they worked did not live when subjected to temperatures of 5°–14° F. unless the plants were given "special attention." By subjecting some of them to a special pre-wintering treatment, the plants were made to survive −4° F. It was concluded that the only means of increasing the resistance to low temperatures was by cross-breeding it with resistant types such as Origan, a variety of guayule selected for use in Russia.

BENEDICT (1) observed that potted field-grown transplants, variety 593, were killed at approximately 20° F., while yet in an unhardened condition. Even after hardening for 2 months at temperatures close to 20°, the plants were not able to withstand 15° F. Plants grown in soil with a low moisture content were no more resistant. A few young

[1] Physiologist; Special Guayule Research Project, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U.S. Department of Agriculture, Beltsville, Maryland.

seedlings survived 15° overnight, but were killed by exposure to 10°. BENEDICT concludes that one-year-old plants of guayule of the variety used cannot be hardened sufficiently to withstand temperatures of 10° F. or below without some injury; but he suggests that there may be other cultivated varieties, or some wild plants, which are less susceptible to frost injury than those tested.

This paper reports experiments undertaken for the purpose of comparing different ages and varieties of guayule plants with respect to their ability to withstand low temperatures, and in addition to observe the effect of frost injury on their rubber content.

Methods

Seeds of several selections or varieties were planted under comparable conditions on the same date. These included nos. 406, 593, A-5058, and A-4278. The young plants were later potted, and part of them were kept in a greenhouse during the summer and fall so that they would produce relatively succulent growth, while the remainder were kept outdoors. All four varieties were subjected to the same conditions, irrespective of the locations where they were grown. As the outside plants hardened off during the fall and winter, repeated tests were made with both greenhouse and outside plants to compare their resistance to low temperature injury. In some instances the rubber content of frost-injured plants was compared with that of uninjured plants. In addition, similar experiments were carried out with more mature potted and field-grown plants.

In general, three methods were used to test the hardiness of guayule: (*a*) potted plants were subjected to controlled temperatures for known periods of time; (*b*) potted plants were subjected to prevailing outdoor temperatures; and (*c*) unprotected and protected field plantings were subjected to prevailing outdoor temperatures. Six plants of a kind were used for each test, and the experiments were generally repeated several times.

Results

RESISTANCE OF SUCCULENT COMPARED WITH THAT OF NONSUCCULENT PLANTS.—Seedling plants of varieties 406, 593, A-4278, and A-5058 produced very succulent growth under greenhouse conditions. In contrast, plants of the same varieties and age grown outdoors produced a sturdy woody type of growth. Neither the greenhouse nor the outside plants had been subjected to temperatures which would appreciably increase their frost resistance prior to the date of these early experiments.

Tolerance to low temperatures was determined by subjecting the plants to controlled temperatures for a period of 18 hours. At the beginning of the experiments the plants experienced a sudden drop in temperature to approximately 50° F., since they were moved during the afternoon from prevailing greenhouse or outside temperatures directly to the desired controlled temperature. After cold treatment, they were placed at 50°–60° F. in diffused light until the soil reached air temperature again. Results of this and a similar experiment using only woody plants are given in tables 1 and 2. There were no important varietal differences in the resistance of unhardened seedlings to prolonged cold treatments. Succulent plants were severely injured at 24°, while woody plants were injured approximately the same amount by exposure to 20° F.

In another experiment, the temperature of the air surrounding unhardened

but woody outdoor seedlings was gradually decreased from 63° at 10 A.M. to 24° F. at 4 P.M. and held at 24° F. for a period of 5 hours. During this interval the soil temperature decreased from approximately 60° to 23°. The air temperature was gradually increased to 70°, and finally the plants were returned to the greenhouse. Examinations 2 months later showed that all plants had been killed. It was concluded that when limited to a relatively short period of time, the rate of cooling—preceding exposure to low temperatures—did not appreciably alter the resistance of the plant to injury.

TABLE 1

RESISTANCE OF SUCCULENT AS COMPARED WITH LESS SUCCULENT GUAYULE SEEDLINGS TO LOW TEMPERATURES. PLANTS EXPOSED TO CONTROLLED TEMPERATURES FOR 18 HOURS. RESPONSES EXPRESSED AS DEGREE OF INJURY TO LEAVES AND BRANCHES BASED ON SIX PLANTS FOR EACH TREATMENT. OBSERVATIONS MADE DURING 6-WEEK PERIOD FOLLOWING TREATMENT

Variety	Temperature (° F.)		
	31°	28°	24°
	Succulent greenhouse plants		
A-4278	None	Slight*	Severe
406	None	Slight	Severe
A-5058	None	Slight	Severe
593	None	Slight	Severe
	Woody outdoor plants		
A-4278	None	None	None
406	None	None	None
A-5058	None	None	None
593	None	None	None

* Slight: Leaf injury; branches not injured sufficiently to result in appreciable set-back in growth. Moderate: Many leaves killed and some branches injured for 1–3 inches back from tip. Severe: Most leaves killed; branches injured back to main stem, so that plants would suffer severe set-back even though they survive; often associated with root injury.

EFFECT OF DURATION OF COLD TREATMENT ON SEEDLING PLANTS.—Potted seedling plants, variety 593, grown outdoors, were subjected in groups of six to 24° for 4, 8, 12, and 16 hours, respectively. The minimum soil temperature in the case of each respective group was 32°, 30°, 30°, and 27° F. Exposure to an air temperature of 24° for 4 hours and to a minimum soil temperature of 32° resulted in only slight leaf injury. Exposure to an air temperature of 24° for 16 hours and a minimum soil temperature of 27° resulted in leaf injury, which did not appreciably inhibit growth of the seedlings. In another experiment, seedlings of variety 406 were subjected to a temperature of 22° for 4, 8, 12, and 16 hours (table 3).

TABLE 2

RESISTANCE OF TOPS AND ROOTS OF WOODY OUTDOOR GUAYULE SEEDLINGS TO LOW TEMPERATURES. RESPONSES EXPRESSED AS DEGREE OF INJURY BASED ON SIX PLANTS FOR EACH TREATMENT. OBSERVATIONS MADE DURING 6-WEEK PERIOD FOLLOWING TREATMENT

Variety	Temperature (° F.)					
	27°		20°		16°	
	Tops	Roots	Tops	Roots	Tops	Roots
593	None	None	Severe	Severe	Killed	Killed
A-4278	None	None	Severe	Severe	Killed	Killed
406	None	None	Severe	Severe	Killed	Killed
A-5058	None	None	Severe	Moderate	Killed	Killed

It is concluded that unhardened seedlings might be expected to withstand long exposures to a temperature of 25°, provided the soil temperature did not range below 27° F. Exposure to temperatures as low as 22° for 8–12 hours or more, however, resulted in severe injury to unhardened seedlings.

FROST RESISTANCE OF MORE MATURE PLANTS.—In preliminary experiments, unhardened potted plants 15–18 months old withstood overnight exposure to a

temperature of 20° F. To study the effect of lower temperatures, hardened plants of the same age, grown outdoors, and also unhardened greenhouse plants, were exposed to controlled temperatures of 18°, 14°, and 10° for a period of 18 hours.

TABLE 3

FROST INJURY OF GUAYULE SEEDLINGS AS RELATED TO DURATION OF EXPOSURE TO LOW TEMPERATURES. RESPONSES EXPRESSED AS DEGREE OF INJURY BASED ON SIX UNHARDENED OUTDOOR SEEDLINGS FOR EACH TREATMENT. TREATMENTS APPLIED OCTOBER 16

Exposure time (hours)	Temperature (° F.)		Plant response December 16
	Air	Soil minimum	
4.....	22	32	Only slight leaf injury
8.....	22	30	Only slight leaf injury
12.....	22	28	Severe injury
16.....	22	25	Killed

The outdoor plants had previously been exposed to moderately cool nights for 3 weeks, during which time the minimum night temperature was 35° and the average minimum night temperature 46° F. (table 4). This experiment was repeated three times with similar results, which illustrated that unhardened potted plants 15-18 months old were no more resistant to frost injury than were young seedlings 6–8 months old. The experiment demonstrated, however, that the upper stems and branches of the older plants could be hardened so that they would withstand exposure to 10° for a period of 18 hours with only slight injury. In contrast to the above-ground parts, the roots of these hardened plants were injured so severely, even at an air temperature of 18°, that death resulted.

Results of a subsequent experiment using potted plants of different varieties and ages also demonstrated that the roots of guayule were relatively sensitive to low soil temperatures. Seedling plants 8 months old, including varieties 593, 406, A-4278, and A-5058, were grown at low temperatures in a coldframe during the fall to increase their resistance to frost injury. More mature plants, variety 593, 15–18 months old, were also grown under the same conditions. On December 21, these hardened plants, plus some unhardened greenhouse ones (variety 593, 15–18 months old), were exposed to prevailing outside temperatures. On January 3 all plants were transferred to the greenhouse and examined for frost injury. The stems of the more mature plants became acclimated to low temperatures and were thus able to withstand exposure to 4° F. (table 5), but the roots were severely injured under these

TABLE 4

FROST INJURY OF SUCCULENT GREENHOUSE GUAYULE PLANTS 15–18 MONTHS OLD, COMPARED WITH THAT OF MORE WOODY PLANTS OF SAME AGE GROWN OUTDOORS. RESPONSES EXPRESSED AS DEGREE OF INJURY TO THAT PART OF PLANT ABOVE SOIL LEVEL. RESULTS BASED ON SIX PLANTS FOR EACH TREATMENT. DURATION OF EXPOSURE 18 HOURS

Temperature (° F.)	One week after treatment		Two months after treatment	
	Greenhouse	Outside	Greenhouse	Outside
18.....	Severe	Slight	Killed	Killed
14.....	Very severe	Slight	Killed	Killed
10.....	Very severe	Slight	Killed	Killed

conditions. Stems of the younger plants failed to become acclimated sufficiently to withstand an exposure to the minimum prevailing temperatures.

EFFECT OF DURATION OF COLD TREATMENT ON MORE MATURE PLANTS.—In pre-

TABLE 5

FROST RESISTANCE OF GUAYULE SEEDLINGS AS COMPARED WITH MORE MATURE PLANTS. RESULTS EXPRESSED AS DEGREE OF INJURY BASED ON SIX PLANTS PER TREATMENT

VARIETY AND AGE OF PLANTS	MINIMUM TEMPERATURE (° F.)				OBSERVATIONS
	Six weeks preceding experiment		During test		
	Range	Mean	Range	Mean	
406, 8 months	21–55	35	4–32*	18	Killed
593, 8 months	21–55	35	4–32	18	Killed
A-4278, 8 months	21–55	35	4–32	18	Killed
A-5058, 8 months	21–55	35	4–32	18	Killed
593–15, 18 months (outside)	21–55	35	4–32	18	Roots killed; tops not injured
593–15, 18 months (greenhouse)	36–60	43	4–32	18	Killed

* Minimum temperature of 4° prevailed on December 24; on all other days it remained between 8° and 32° F.

liminary experiments it was observed that unhardened greenhouse plants 15–18 months old were killed by exposure to 0° F., even though the duration of exposure was only 3 hours. Additional experiments were made, using plants 15–18 months old of varieties 593 and A-5058. These plants had grown in pots under comparable conditions outdoors during the fall at minimum night temperatures of 32°–58° F. An average of 39° F. prevailed for the 3 weeks preceding the experiment.

On November 8 the plants were subjected to controlled temperature conditions as indicated in table 6. Varietal difference was observed with respect to frost resistance, since hardened plants, variety 593, were killed by exposure to an air temperature of −5° for a period of only 3 hours, while plants of variety A-5058 survived with only slight root injury (table 7). In general, the results indicate that even the most hardy of the potted plants were unable to withstand exposure to an air temperature of −5° and a soil temperature of 16° F. for longer than a few hours.

TABLE 6

TEMPERATURE CONDITIONS UNDER WHICH FROST RESISTANCE OF PRE-HARDENED GUAYULE PLANTS 15–18 MONTHS OLD WERE TESTED

TIME	HOURS OF TREATMENT	TEMPERATURE (° F.)	
		Air	Soil
Nov. 8: 10 A.M.	0	− 5	.....
1 P.M.	3	− 5	16
4 P.M.	6	− 5	5
7 P.M.	9	− 5	− 4
7 P.M. to 8 A.M.	.....	35	32
Nov. 9: 11 A.M.	.....	40–50	32
1 P.M.	.....	50–60	32
3 P.M.	.....	50–60	54
4 P.M.	.....	50–60	59

WINTER HARDINESS OF FIELD-GROWN PLANTS.—Some plants of variety 593, 8–10 months old, were left unprotected in the field during the fall and part of the

winter, while others of the same age and variety were completely covered with pine needles to maintain a relatively high temperature in the air and soil immediately surrounding the plants. Other similar plants were covered with a wooden box to eliminate light. All plants remained under these conditions for 3 weeks previous to a relatively cold period of 12 days. A week later, the plants were transferred to a greenhouse and examined to determine the extent and location of injury.

TABLE 7

VARIETAL DIFFERENCE IN RESPONSE OF GUAYULE PLANTS TO TEMPERATURE CONDITIONS LISTED IN TABLE 6. RESPONSES EXPRESSED AS DEGREE OF INJURY BASED ON GROUPS OF SIX PLANTS PER TREATMENT

VARIETY	DURATION OF EXPOSURE (HOURS)		
	3	6	9
593.....	Killed	Killed	Killed
A-5058...	Only fibrous roots injured; stems uninjured	Killed	Killed

It was possible to harden the aboveground portions of field-grown plants (table 8). In such condition the stems and small branches of field plants withstood a minimum temperature of 6° and an extended cold period during which the minimum temperature declined to 15° or below for a period of 12 consecutive days. The roots of field plants failed to survive these conditions, however, even though the minimum soil temperatures were not below 26.6° F. The absence of light during a period of approximately 5 weeks did not appreciably change the resistance of the plants to frost injury.

The present results indicate that guayule can readily be protected against temperatures near 0° F., and probably subzero weather, by means of a mulch. Such protection may be of interest in connection with experimental plantings.

EFFECT OF INJURY ON RUBBER CONTENT.—Plants of different ages were treated. One group was kept in a greenhouse having a minimum temperature of 40° and a usual daily range of 45°–65° F. Another group was kept in a coldframe

TABLE 8

RESPONSE OF FIELD-GROWN GUAYULE PLANTS TO LOW TEMPERATURE. DEGREE OF INJURY BASED ON GROUPS OF FIVE PLANTS PER TREATMENT

CONDITION	MINIMUM TEMPERATURE (° F.) FOR 3 WEEKS PRECEDING LOW PERIOD	LOW TEMPERATURE PERIOD (DEC. 14–25)			INJURY OBSERVED JAN. 3
		Minimum air temperature		Minimum soil temperature at depth 3″	
		Range	Mean		
Unprotected....	15–38	6–15	11	26.6	Severe root injury; no apparent top injury
In darkness.....	21–42	10–19	14	.....	Severe root injury; no apparent top injury
Covered with mulch.....	21–53	18–27	22	35.6	No apparent root or top injury

at temperatures generally ranging between 25° and 45°, with a minimum of 21°, which caused no apparent injury. The third group was grown in a cold-frame at approximately the same temperatures, except that the plants were exposed to a minimum of 6° during one night. These were severely frost-injured. Subsequent analyses indicated that the rubber content of the frost-injured plants was not significantly different from that of those uninjured (table 9).

TABLE 9

EFFECT OF FROST INJURY ON RUBBER CONTENT OF GUAYULE. FIGURES REPRESENT PERCENTAGE RUBBER IN DRIED STEMS OF SEEDLING PLANTS ASSAYED WITHIN 2 MONTHS AFTER TREATMENT. TEMPERATURES REPRESENT MINIMUM TO WHICH EACH GROUP WAS EXPOSED

Plant no.	Plants 8 months old (° F.)			Plants 15 months old (° F.)		
	40°	21°	6°*	40°	21°	6°*
1	2.4	3.6	2.0	4.8	3.9	3.7
2	2.4	2.2	2.5	4.0	4.3	3.5
3	3.8	3.1	2.2	4.8	2.6	3.2
4	2.4	2.8	2.6	1.8	3.5	5.4
5	2.1	2.7	4.7	3.8	4.5	2.0
Average	2.6	2.9	2.8	3.8	3.8	3.6

* Plants exhibited symptoms of very severe frost injury.

In a similar experiment two groups of five plants each were grown under field conditions. Plants of one group were covered with a mulch, while the others were left unprotected. The former, subjected to a minimum of 20° F., showed no evidence of frost injury. The latter were exposed to a minimum of 6° F., which resulted in the death of root and stem tissues of each plant up to approximately 1 inch above the ground level. The leaves were also killed, but the upper portions of the stem and main branches were uninjured. Rubber analyses made within 2 months after treatment showed an average content of 3.3% for the protected and 3.7% for the unprotected plants.

In another experiment, sixteen uniform unhardened plants grown in a greenhouse with a daily range of approximately 45°–65° and a minimum of 40° were divided into two equal groups. One was subjected to 18° F. for a period of 18 hours, the other was kept at room temperature. Roots, leaves, and small branches of the cold-treated plants showed evidence of frost injury. Analyses of the individual plants made approximately 1 month later showed no significant difference between the percentage of rubber in the injured as compared with that of the uninjured plants, the means being 4.4 and 4.6, respectively.

Conclusions

On the basis of the present experiments, seedlings—and also more mature plants of the type generally grown for rubber—did not withstand temperatures of 15°–20° F. for more than a few hours at a time without injury, unless they had previously been acclimated to low temperatures. The reason for this relatively high critical temperature seems to lie mainly in the fact that roots of guayule are subject to injury when exposed to a soil temperature of 26°–28° F. for a period of only 8–10 hours. Like many other kinds of woody plants, the stems and leaves of the more nearly mature ones can be readily acclimated to low air temperatures by subjecting them to a hardening period of several weeks. In a winter-hardened condition, the stems of guayule transplants withstood repeated and prolonged exposures to temperatures ranging 5°–10° above zero, and in plants of variety A-5058, the stems survived an

exposure of 3 hours at 5° below zero with no apparent injury. There was no evidence that the resistance of roots to low temperature increased as the result of pre-hardening. However, most soil temperatures used in these tests were relatively low and caused severe injury. It is possible that slight differences in the resistance of roots to low soil temperatures would have been apparent had more moderate soil temperatures been used.

Sharp differences were observed when the frost resistance of two varieties of older plants were compared, and some variation was noted in the resistance of individual plants within the variety A-5058. It is therefore apparent that field plants may vary in their ability to withstand exposure to low temperatures and that some variation in this respect might be expected among the individuals in some of the known varieties or selections. On this basis, a selection and cross-breeding program would offer a promising means of increasing the average winter hardiness of guayule plants.

Summary

1. Woody outdoor seedlings of guayule not yet unacclimated to low temperatures survived only slightly lower temperatures than did very succulent greenhouse plants of the same age.

2. The rate of cooling, preceding an extended exposure to low temperatures, did not appreciably alter the resistance of the plant to frost injury.

3. Unhardened seedlings of four varieties withstood prolonged exposure to a temperature of 25° but were severely injured as the result of an 8–10-hour exposure to 22° F.

4. In most instances, the roots were severely injured by prolonged exposure to soil temperatures of 26°–28° F. As an exception, roots of plants of selection A-5058 survived a short exposure (less than 3 hours) to a soil temperature of 16° F.

5. Stems and leaves of transplants were readily acclimated by means of low temperature hardening treatments, so that they withstood repeated exposure to temperatures ranging 5°–10° above zero. Stems of hardened plants of selection A-5058 withstood a 3-hour exposure to a temperature of 5° below zero. The roots of these plants were slightly injured but the plants survived.

6. Based on a limited study of four selections, plants of A-5058 were most resistant to frost injury.

7. Experimental field plantings can readily be protected from temperatures as low as 4° F., and probably lower, by means of an adequate mulch.

8. The rubber content of plants severely injured by frost was not significantly different from that of comparable uninjured plants during a period of 1–2 months after treatment.

All rubber determinations were made by Mr. R. L. Holmes.

Bureau of Plant Industry, Soils and Agricultural Engineering
Beltsville, Maryland

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STUDIES OF DEVELOPMENT IN LARKSPUR. I. FORM SEQUENCE IN THE FIRST TEN MATURE LEAVES

SPENCER W. BROWN

Introduction

Homologous organs formed in series offer advantages to the student of growth and developmental relationships in plants. Since individual organs, as successive leaves borne by a growing stem, may be inspected or later removed without injury to the organism as a whole, only a small number of plants need be grown to secure a relatively great number of measurements. Thus a single organ complex can be analyzed through all growth phases without sacrificing material. In addition, definite intervals of physiological time are available, especially useful when time is to be cancelled out, as in studies of allometry.

WHALEY (6) found that the size of organ produced could be directly correlated with the size of the apical meristem at any time during development. From the four or five measurements he gave for the entire active growth period, it is impossible to tell whether the ontogeny of the apical meristem follows a parabolic or a sigmoid developmental pattern, but the latter seems more likely. ABBE, RANDOLPH, and EINSET (1) were able to show that increase in width of corn leaves numbers 6 through 12 can be attributed to a corresponding increase in size of the growing point. Although detailed data are given by these writers to show the parallel growth patterns of successive leaves, analysis is lacking of the changes in size as a function of time of either the growing points or the mature leaves. It was obviously necessary to establish first a relationship between apex and developmental pattern which would then allow changes in adult leaves to be interpreted, at least in part, as reflections of underlying changes in growing points.

The present report is intended to provide preliminary information on factors regulating leaf development in larkspur (*Delphinium ajacis* L.) by an analysis of changes in the first ten mature leaves of several characters—number of points, length, area, and area/length.

MATERIAL AND METHODS.—Cultivated double-flowering larkspurs were grown in the greenhouse at Athens, Georgia, during the winter 1943–44, under approximately uniform conditions. The seedlings were transplanted to 2-inch pots at about the second-leaf stage, and at about the five-leaf stage were carefully reset in 5-inch pots. Although definitely not isogenic, the material seemed fairly uniform in regard to the characters employed in the present study.

Counts of the number of points per leaf (fig. 1) were made shortly after the young leaves had grown out completely from the terminal bud. Of course, the number of points remained the same during later development of individual leaves. Leaves were later removed from the plants for measurement when they began to turn a lighter shade of green, or yellowish at the margins, and it was apparent that their usefulness to the plant was probably finished. By the time the second or third leaf was removed, the tenth seemed already to be completely matured. The leaves were pressed lightly for a few hours, and then silhouettes obtained on photosensitive paper as long as the paper was available. Later it became necessary to use the leaves themselves in projection. Tracings were made of projections at five or ten diameters; from these, area and length measurements were made with planimeter and steel rule, respectively. Length measurements are of the blade only and do not include the petiole (fig. 1, leaf 1).

After the point number counts were made, some of the leaves were injured, either by handling or by insects. If more than one or two leaves were injured, the plant was completely eliminated from all but the point counts which had been made previously. The point counts plotted against length and area are from the same plants on which the length and area measurements were made. In all cases where there was a variation in total number from leaf to leaf (for example, N = 26–28), the same group of plants (here twenty-eight) was used throughout.

Developmental sequences

The first four to six larkspur leaves are borne in a rosette, later leaves coming above successively longer and longer internodes. The longest internodes occur between the twelfth or thirteenth leaf and the inflorescence. Apparently there is a decided relationship between length of petiole and position at which the leaf is borne; at first the larger leaves have longer petioles, while those four to six internodes above the rosette begin to have shorter petioles the longer the internodes. No such influence of position, other than order in series, could be determined in regard to the leaf character employed here, either by inspection of the plants themselves or by the curves obtained.

The larkspur leaf is divided basically on a trichotomous pattern. All the first leaves, after the cotyledons, had at least three sections, and these three basic sections are retained in all subsequent, more incised leaves (fig. 1). Within each of the three basic sections, subsequent trichotomous division in the older leaves may be decidedly imperfect. In leaf 3, figure 1, it may be seen that, although the middle section (*M*) and one side (*Sa*) are divided again just three times, in the other side (*Sb*) the lowermost of the three components is split again into two points. In the older leaves, patterns even more divergent from the simple trichotomous type are found.

Although the total number of points progresses smoothly from leaf to leaf (fig. 2*T*), it was apparent that this increase did not maintain the same proportion of points between the middle section and the two sides. When the average of the points per side is plotted alongside the average of the points per middle section against leaf number (fig. 2*S* and *M*, respectively), the change in ratio is clearly obvious. The shape of curves *S* and *T* of figure 2 seems to suggest a sigmoid sequence with upper inflections beginning at leaf 8.

Logarithmic plotting of the average number of points for the sides against the average for the middle section yields an even more irregular curve than that for either of these two variables plotted against leaf number (fig. 3). The shape appears to be doubly sigmoid, the lower section from leaf 1 to leaf 7, the upper from leaf 7 to leaf 10. A straight line drawn by inspection yields a slope or *k* value in the HUXLEY (2) equation for relative growth[1] of 1.23. Table 1 gives the nine progressive *k* values calculated from the logarithms of the averages for the slopes of the straight lines connecting leaf 1 with leaf 2, leaf 2 with leaf 3, etc. In view of the recent evidence showing that the consequences of sigmoid growth may (4) but not necessarily (3) lead to wide differences from the linear of relative growth curves plotted logarithmically, the *k* values calculated for the individual sections of the curve must not be considered as actual changes in the HUXLEY relative growth constant or in

[1] $y = bx^k$.

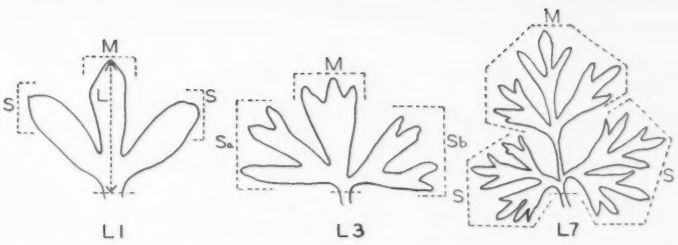
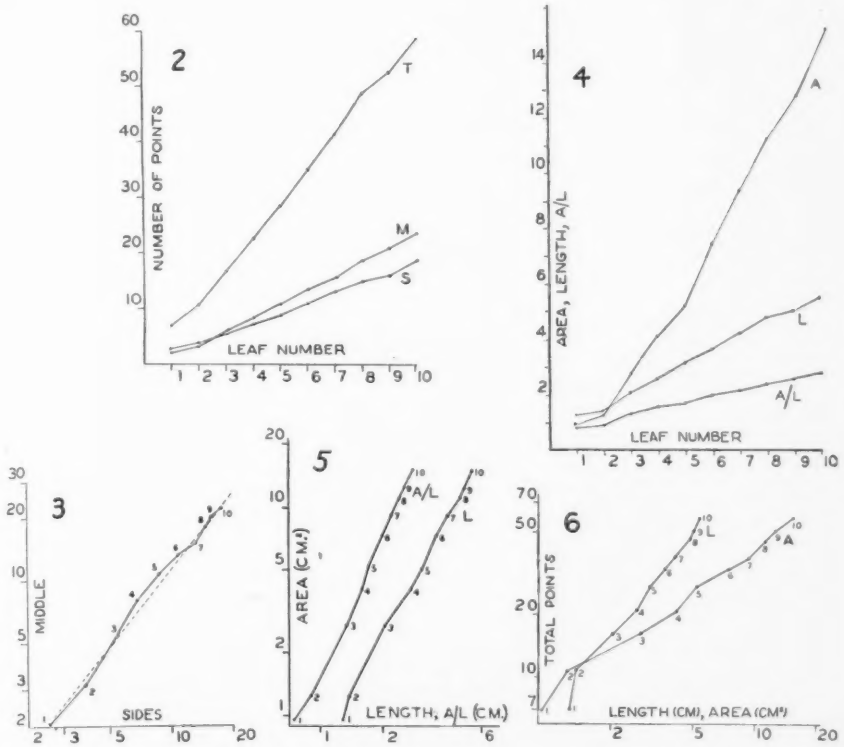


FIG. 1.—Three leaves from plant no. 32a16, redrawn from projection outlines: leaf 1, $\times 2\frac{1}{2}$; leaf 3, $\times 1\frac{1}{4}$; leaf 7, $\times \frac{5}{6}$. *S*, points included in counts per side (*Sa*, *Sb*, see text); *M*, points included in counts per middle; *L*, length measurement.



FIGS. 2–6.—Variation and relative development of several characters in leaves 1–10. All points connected by straight lines. Fig. 2, number of points: *T*, total; *M*, middle; *S*, side; against leaf number; N = 34–36 for each leaf number. Fig. 3, points per middle against points per side; logarithmic; leaf numbers given along curve; N = 34–36 for each leaf number. Dotted line is average slope from inspection (slope = 1.23). Fig. 4, area, length, and area/length against leaf number; N = 26–28 for each leaf number. Fig. 5, area against length and area/length; logarithmic; leaf numbers given along curve; N = 26–28 for each leaf number. Fig. 6, total points against length and area; logarithmic; leaf numbers given along curve; N = 26–28 for each leaf number.

other growth constants not considered here.

The three other characters studied—area, length, and area/length—also suggest, but less definitely, a sigmoid type of development when plotted against leaf number (fig. 4). When area/length and length are plotted against area on a log-log grid (fig. 5), the relative growth curve takes on a more decided sigmoid aspect. The steep slopes, approximating 2, of both these curves are to be expected when growth in two dimensions is plotted logarithmically against growth in one. The more emphatic sigmoid trends observable in the logarithmic plots (figs. 3, 5) indicate that, had it been possible to utilize absolute rather than physiological time intervals for arithmetic plotting, these curves themselves would have been more obviously sigmoid.

TABLE 1

INDIVIDUAL k* VALUES BETWEEN SUCCESSIVE LEAVES

Leaf numbers	k
1 and 2	1.08
2 and 3	1.47
3 and 4	1.58
4 and 5	1.27
5 and 6	1.01
6 and 7	0.78
7 and 8	1.48
8 and 9	2.32
9 and 10	0.60
Unweighted average	1.29

* In the HUXLEY (2) relative growth equation, $y = bx^k$.

Logarithmic plotting of area and length against total number of points (fig. 6) yields the interesting observation that the number of points increases directly with the area and exponentially with the length, and thus exponentially with the circumference or any other linear dimension. Therefore, the number of points must be regarded as expressing the number of segments into which the area is divided, rather than segments resulting from splits engendered by increase in circumference. If a certain rather large minimal increase in area is required before a new segmentation will occur, a broken relative growth curve would be expected, as was actually found. In this fashion, the shape of the curves in figure 6 may be accounted for without assuming repeated radical changes in growth constants for which there is no apparent physiological basis.

Discussion

The most interesting observation to be made on the material reported here is that the fully developed leaves of larkspur bear a relationship to one another in the characters analyzed typical of developmental sequences in single organs. There is no a priori reason that this form of development would be followed, and much additional work must be done before it can be assumed generally true. The best interpretation at hand is that the growing point itself changes in a regular fashion, and that this regular change is reflected in the mature leaves. Thus an analysis of the curve of changes in growing point size is indicated as a basis for further understanding of problems of this type.

The field to be analyzed may be conveniently divided into three major aspects: (1) developmental sequence in the growing point; (2) development of the homologous organs formed from the growing point; (3) maturation of the homologous organs.

Studies combining data relevant to all these aspects will be necessary to explain sufficiently the observations currently reported. Only the development of the homologous organs has heretofore been

analyzed to a satisfactory extent. ABBE *et al.* (1) have shown that development in homologous organs is parallel. WHALEY (6) states that organ size may be directly correlated with meristem size at any stage during development; however, no data were given which could be used to distinguish complete dependence of organ on the growing point from at least partial autonomy of the individual organ in length of time of development. That the leaf may have considerable autonomy in some respects is indicated by WHALEY'S (7) analysis of the change in shape during individual development of leaves of *Tropaeolum* arising from presumably mature meristems. Consequently only two factors, environmental changes and differences in the completion of maturation, prevent the mature leaves from being direct reflections of the changes in growing point size. The effects of environmental changes are obvious. For example, leaf 1 may be completely matured, while leaf 6 suffers from the effect of an environmental change which does not affect leaf 8 because it has not yet appeared.

SINNOTT (5) has shown that difference in fruit shape between two races of gourds, both continuing along the same course of heterogonic development, is due simply to the attainment of a larger size by one race than by the other. Such size differences, or differences in length of developmental period, would yield variation in the homologous organ series not traceable to the growing point. Although not likely in the case of number of points (the same in the young as in the mature leaves), and consequently of the other characters showing a direct relationship to point number, effects of this sort might possibly be operating in all the characters analyzed here. Finally, if longer developmental periods of the individual leaves were staggered symmetrically, the influence on the shape of the curve would not be obvious without biological criteria. The simplest way of solving this last problem is again by a study of growing point changes, comparing them with the mature leaf sequences.

A final point of interest is the bearing of studies on the developmental relationships of leaves to a solution of the stem-leaf versus the shoot-complex interpretation of plant structure. As yet biologists have devised no satisfactory criteria for measuring degree of differentiation. Yet in deciding whether a given heterogeneous structure should be regarded as composed of two distinct organs (here leaf and stem) or of a single organ of two parts (here shoot-complex), the solution rests ultimately on the interdependence of growth and differentiation in the two parts. If an organism is to be symmetrical, capable of functioning as an integrated whole, each part must normally bear a definite developmental relationship—within rather broad limits—to every other part. The technique of relative growth measurements seems to offer the best method for a comparative study of the magnitude of developmental interdependencies.

Summary

1. Mature leaves of larkspur, for the aspects studied, bear a relationship to one another typical of growth sequences in single organs.

2. Slightly sigmoid curves were obtained when the characters (number of points per side, per middle, total, and area, length, and area/length) were plotted against physiological time intervals (leaf number).

3. Log-log plotting of some characters against others yields relative growth re-

lationships expected from sigmoid developmental sequences.

4. The problem of the developmental factors underlying these relationships is briefly analyzed.

5. The number of points is in direct relationship to the area of the leaf, indicating that incision must be regarded as a segmentation of the area rather than a splitting in from the circumference.

6. The use of homologous organ series in problems of relative growth and the use of relative growth techniques in estimating degree of differentiation are discussed.

The writer is indebted to the Horticulture Department for greenhouse facilities.

DIVISION OF BIOLOGICAL SCIENCE
UNIVERSITY OF GEORGIA
ATHENS, GEORGIA

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APPLICATION OF GROWTH-REGULATING SUBSTANCE IN AEROSOL FORM, WITH SPECIAL REFERENCE TO FRUIT-SET IN TOMATO

CHARLES L. HAMNER,[1] HAROLD A. SCHOMER,[2] AND PAUL C. MARTH[3]

Introduction

Many methods have been employed in applying growth substances for obtaining parthenocarpic fruit-set on tomatoes, including spraying the flowers with lanolin emulsions containing the substances, smearing the styles with lanolin-growth substance paste, or applying vapors of growth substances to the flowers (**3, 6, 8**). HOWLETT (**5**) has effectively used lanolin emulsions as carriers in increasing fruit-set, size, and quality. The most effective growth substance reported by him was indolebutyric acid. He has recommended that the treatment should follow pollination and be near the end of full bloom. This method does not result in the production of seedless fruit but increases fruit-set, size, and quality. An atomizer is employed to spray each individual flower; hence large-scale operations may be difficult. MITCHELL and WHITEHEAD (**7**) found that under greenhouse conditions β-

[1] Assistant Plant Physiologist, [2] Associate Plant Physiologist, [3] Associate Plant Physiologist; Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U.S. Department of Agriculture, Beltsville, Maryland.

naphthoxyacetic acid vaporized on a hot plate resulted in the development of 50–98% seedless fruits. Plants of the Marglobe variety were successively fumigated as the earliest flowers of each cluster opened.

Recently a new method of hormone application has been used (4), based on the aerosol principle devised by GOODHUE (2) for applying insecticides. In the initial experiments, β-naphthoxyacetic acid applied by this method was very effective in stimulating the production of seedless fruits and in increasing the number set. This paper reports further studies of the aerosol method, using β-naphthoxyacetic acid and several other growth substances, tested at many concentrations and under various conditions.

The method of aerosol production generally employed in these experiments is a simple one. The growth-regulating substance is first dissolved in some compound in which it is readily soluble and then added to a highly volatile liquefied gas. If the substance is soluble in the liquefied gas, it may be added directly to it. In either case, this mixture of liquefied gas, growth substance, and carrier solvent must be held under pressure in a container which is equipped to release the mixture as a very fine mist. After such release, the carrier promptly volatilizes and leaves the growth substance suspended in the atmosphere as an exceedingly finely divided liquid or solid.

In developing the method, it was necessary first to find a solvent for the naphthoxyacetic acid having a boiling point so high that, when sprayed, the acid would remain in solution in the finely dispersed droplets rather than fall out of solution as a solid or as dry crystals suspended in the air. Cyclohexanone (boiling point 155° C.) was finally determined upon as a suitable solvent. Others—such as acetone and carbitol—were tried, but cyclohexanone was selected because it readily dissolved many growth-regulating substances and it had also proved very effective in insecticidal aerosols.

It was necessary also to secure a liquefied gas readily miscible with the cyclohexanone and solution of growth substance and yet one which after release would evaporate almost instantaneously. Methyl chloride[4] (boiling point −10.57° F.) and dichloro-difluoromethane (Freon) (boiling point −21.7° F.) were first tested, but neither was miscible with this particular growth substance dissolved in cyclohexanone. Methyl ether (dimethyl ether) (boiling point −9.6° F.), however, proved a satisfactory solvent for cyclohexanone and the acid, and was miscible with the cyclohexanone.

The vapor pressure of suitable liquefied gases is sufficient to disperse the solution into fairly small droplets as it emerges from the spray nozzle. The droplets are probably still further subdivided by the violent boiling of the liquefied gas remaining in the droplets. By the time all the gas has completely disappeared from the droplets, most of them are so small that they settle out of the air very slowly.

The initial testing of the solubilities of various growth-regulating substances in these liquefied gases was done by L. D. GOODHUE of the Bureau of Entomology and Plant Quarantine. For this purpose two cylinders (fig. 1) were designed (2). The pure liquefied gas from a large stock container was transferred to the small steel cylinder (fig. 1*A*) by means of a short loading tube. The glass test-tube of the second cylinder (fig. 1*B*) was then removed by unscrewing the plug from the lower end. Into this tube the desired amount of growth substance or its solu-

[4] E. I. du Pont de Nemours and Company.

tion was put. The tube was then replaced in the cylinder and tightened into position by means of the screw at the base. The spray nozzle was replaced by a hexagonal nipple, and—with the valve open—the air in the tube was evacuated by means of a suction pump. Following evacuation, the valve was closed and

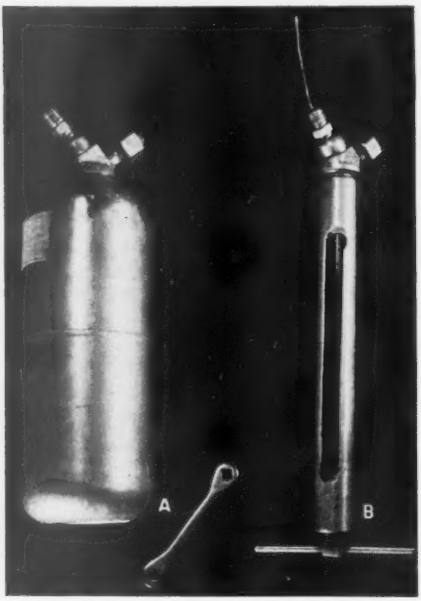


FIG. 1.—Apparatus for aerosol treatments: *A*, steel cylinder with spray nozzle and wrench; *B*, testing tube with fine capillary nozzle.

the two cylinders connected by means of the nipple. The valves were opened on each cylinder, thus allowing the liquefied gas to flow from *A* to *B*. If sufficient solvent failed to move, hot water was applied to *A*, thereby increasing the vapor pressure in that cylinder. Ten grams of solvent was forced into the glass test-tube and the solubility determined. If the substances were insoluble, a cloudy precipitate formed. β-naphthoxyacetic acid, indolebuytric acid, indoleacetic acid, and α-naphthaleneacetic acid are all soluble, at least up to 1% in solutions consisting of 10% cyclohexanone and 89% methyl ether.

In applying the methyl ether-cyclohexanone solution of growth substance as an aerosol to tomato flowers in amounts up to 500 gm., a cylinder similar to that of figure 1*A* may be used. If only a few plants are treated, the cylinder with glass test-tube (fig. 1*B*) is suitable. When using either cylinder, the valve is opened with a wrench (fig. 1), a spray nozzle may be attached (fig. 1*B*), and the amount of solution used in a given time determined by accurately weighing the cylinder before opening and after closing the valve. The nozzle delivery rate is calibrated by this method.

Investigation

EXPERIMENT I: FUMIGATION METHOD OF APPLYING β-NAPHTHOXYACETIC ACID AS AN AEROSOL

The term "fumigation" as used in this paper refers to the use of the aerosol as a vapor treatment, in which the aerosol is released into a closed room in which everything is exposed to the treatment.

For the tests of the effect of fumigation with an aerosol of naphthoxyacetic acid dissolved in cyclohexanone and methyl ether, Pan America tomato plants were grown in 8-inch pots under greenhouse conditions, with temperatures maintained at 65°–70° F. at night and 70°–75° F. during the day. When they were approximately 18 inches tall (fig. 2) and the first flower of the first cluster had opened, ninety-six of the most nearly uniform plants were chosen and forty-five of these placed in a closed room some distance from the greenhouse. They were then treated with aerosol by releasing a mixture supplying 240 mg. naphthoxy-

acetic acid, 21.36 gm. methyl ether, and 2.4 gm. cyclohexanone per 1000 cu. ft. The plants were treated at 4:00 P.M. October 16, 1943, and were taken out of the room at 8:00 A.M. the following day. They were then placed on the greenhouse benches, together with the control plants which had not been treated.

RESULTS.—Five days after treatment the plants that had received the aerosol

FIG. 2.—Maturity of tomato plant at time of first treatment with aerosols.

treatment had 123 fruits (table 1), or an average set of about three fruits per plant, and at this time the largest of them were about $\frac{1}{2}$ inch in diameter (fig. 4). Although the clusters were treated when only the first flower had opened, many of the unopened flowers set fruits, and these enlarged rapidly. The fifty control plants (fig. 3) at this time had set but two fruits. The style persisted much longer on the treated than on the control fruits and showed some enlargement. Likewise some of the petals persisted 3–4 days longer on the treated than on the untreated flowers.

As the fruits ripened they were removed from the plant, weighed, and sliced to determine the relative seed development and general condition. The treated tomatoes ripened earlier and were larger in diameter and heavier than the controls (table 2), and most of them were seedless. In a few, however, the ovules had undergone partial development, the amount varying with the condition of the flower when treated. The flowers that were open at the time of treatment showed greater ovule development than did those treated prior to opening of the flowers.

TABLE 1

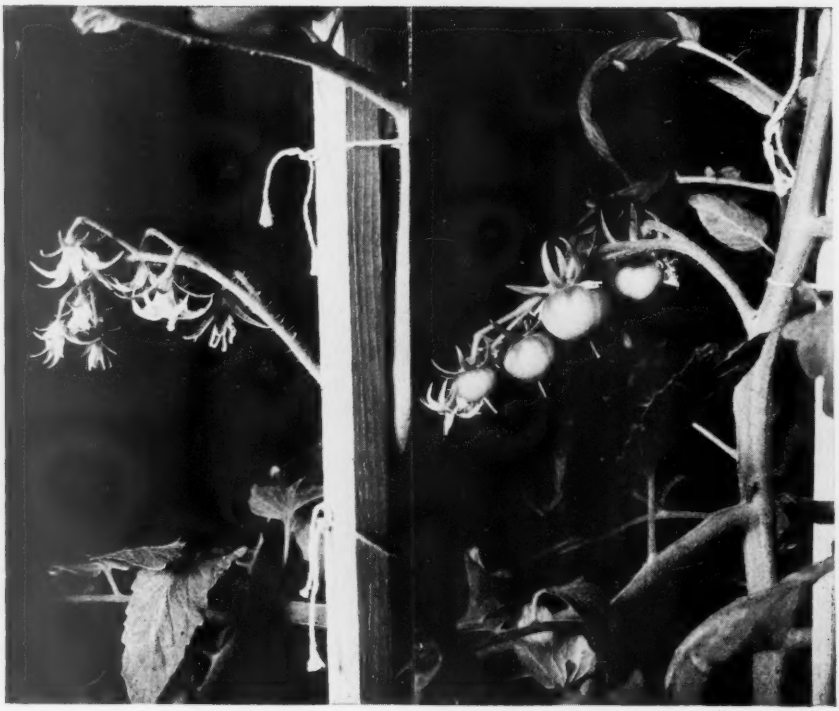
NUMBER OF FRUITS SET ON FIRST CLUSTER OF TOMATO PLANTS, OCTOBER 21, 1943, 5 AND 9 DAYS AFTER TREATMENT BY FUMIGATION WITH β-NAPHTHOXYACETIC ACID AS AEROSOL

OBSERVATION DATE	TREATED		CONTROLS	
	No. of plants	No. of fruits set	No. of plants	No. of fruits set
October 21..	45	123	50	2
October 25..	45	123	50	22

EXPERIMENT II: DIRECT APPLICATION OF AEROSOL

Spraying of the aerosol differs from the fumigation treatment in that the aerosol mixture is sprayed directly at the open flower cluster (fig. 5), necessitating no inclosed chamber or removal of the plants from their original positions. In this respect the operation resembles any ordinary water-spray method.

Three varieties of tomatoes—Pan America, Marglobe, and Bonny Best—were grown under greenhouse conditions during February, 1944. At the time the first cluster of flowers had opened, sixty plants were chosen from each of the varieties and these divided into five lots



FIGS. 3, 4.—Fig. 3 (left), untreated plant 5 days after treatment was administered to plant shown in fig. 4. Fig. 4 (right), 5 days after treatment of cluster with 1% β-naphthoxyacetic acid used as aerosol in fumigation method.

TABLE 2

DATES OF RIPENING AND WEIGHTS OF MATURE FRUITS FROM PLANTS TREATED BY FUMIGATION WITH β-NAPHTHOXYACETIC ACID AS AEROSOL, AND FROM UNTREATED CONTROLS. TREATMENT OCTOBER 16, 1943, WHEN FIRST FLOWER OF FIRST CLUSTER WAS OPEN

Harvested (1943)	Aerosol-treated			Controls		
	Av. wt. of fruits (gm.)	Av. diameter of fruits (inches)	No. of fruits	Av. wt. of fruits (gm.)	Av. diameter of fruits (inches)	No. of fruits
11/27	175.7	3.07	12	130	2.71	1
11/29	187.3	3.00	16	68	2.01	1
11/30	207.0	3.13	5	96	2.83	1
12/1	193.0	3.08	9	165	2.82	3
12/2	211.0	3.14	14	96	2.23	4
12/3	167.0	2.83	15	116	2.45	4
12/4	158.0	2.75	8	111	2.39	5
12/6	151.0	2.68	12	148	2.60	7
12/7	161.0	2.72	5	145	2.59	3
12/9	158.0	2.77	4	120	2.45	11
12/11	154.0	2.79	3	114	2.42	4
12/13	130	2.51	3	110	2.39	13

of twelve plants each. The first three lots received 1% concentrations of 4-chlorophenoxyacetic, naphthoxyacetic, and indolebutyric acid, respectively, in the form of cyclohexanone–methyl ether aerosols. The fourth lot received aerosol without growth substance. The fifth was a control which received no spray treatment.

FIG. 5.—Direct application of aerosol. Steel cylinder held approximately 1 foot from flower cluster, valve then opened for a second, and a fine mist envelops cluster. This is sufficient to insure a high percentage of set.

Each lot of plants was successively taken out of the greenhouse into a well-ventilated room. The number of flowers

and buds on each plant was recorded and the plants lined up in a row. The treatment was applied directly to the individual clusters by holding the cylinder about 1 foot away, releasing the valve, and rapidly spraying each cluster with the vapor. The thirty-six plants in each treatment received a total of 12 gm. of aerosol, or 0.33 gm. of solution containing 3.3 mg. of growth substance per plant. In all probability only a small fraction of this amount was deposited on the clusters, of course. After each respective treatment, the doors and windows of the room remained open to disperse the aerosol before bringing in the plants for the application of any succeeding treatment.

After treatment, the plants were returned to the greenhouse and placed at random on six greenhouse benches, being so arranged that two plants per treatment of each variety were placed on each bench, making a total of thirty plants per bench. The plants were then allowed to grow under greenhouse conditions. Records were taken at weekly intervals of the size of the fruits and the number of fruits set. The fruits were harvested at the late-pink to full-red stage of maturity, at which time the final size, weight, and condition were recorded. The control plants received the same amount of handling, except that no aerosol treatments were given. It was thought that the control plants were jarred enough to permit pollination to take place without hand pollination.

TABLE 3

EFFECT OF GROWTH SUBSTANCES APPLIED AS AEROSOL SPRAY ON FRUIT-SET. CONCENTRATION OF COMPOUND IN AEROSOL WAS 1%; OF CYCLOHEXANONE, 10%; AND OF METHYL ETHER, 89%. TREATMENT FEBRUARY 5, 1944

Treatment	Total no. flowers and buds treated	Total no. flowers open when treated	Total no. fruits set on 2/14/44	Av. diameter of fruits on 2/14/44 (inches)	Total no. fruits set on 2/28/44	Av. diameter of fruits on 2/28/44 (inches)	Av. wt. at harvest (gm.)
Bonny Best							
4-Chlorophenoxyacetic acid	57	27	35	0.7	40	1.6	99.3
Naphthoxyacetic acid	62	27	16	0.7	20	1.9	96.8
Indolebutyric acid	61	26	7	0.3	12	1.4	78.9
Aerosol control	60	27	5	0.4	14	1.5	88.0
Untreated	56	28	14	0.6	15	1.3	78.0
Marglobe							
4-Chlorophenoxyacetic acid	57	26	32	0.7	37	2.0	141.8
Naphthoxyacetic acid	59	27	22	0.7	24	1.8	135.0
Indolebutyric acid	53	24	15	0.5	20	1.8	120.3
Aerosol control	62	27	12	0.4	20	1.7	110.9
Untreated	60	24	18	0.5	17	1.5	139.0
Pan America							
4-Chlorophenoxyacetic acid	53	31	32	0.6	44	2.0	128.6
Naphthoxyacetic acid	58	28	16	0.8	18	2.3	177.0
Indolebutyric acid	67	30	7	0.3	17	1.2	80.5
Aerosol control	53	29	5	0.3	13	1.2	92.3
Untreated	58	23	5	0.3	13	1.4	108.8

RESULTS.—In all three varieties, the flowers that received 4-chlorophenoxyacetic acid at 1% concentration set more fruit than did the flowers receiving

other treatments (table 3). There were in fact more fruits set per plant than there were open flowers at the time of treatment, since many of the unopened buds developed fruit directly. With the chlorophenoxyacetic-acid treatment, a total of ninety-nine fruits had set 9 days after treatment, an average of 2.4 per cluster. Twenty-three days after treatment 121 fruits had set, an average of 3.3 per cluster. On the control plants a total of 37 fruits had set 9 days after treatment, an average of 1.0 per cluster. The fruits from the control plants had an average diameter of 0.44 inch as compared with 0.67 inch for the chlorophenoxyacetic acid-treated fruits. A 1% solution of this acid was injurious, and the leaves developed various deformities. The treated fruits at the time of harvest were in some cases misshapen (fig. 6), many had developed rot, and the placentas of some had failed to develop and fill the carpellary cavities. No seeds were present in any of the treated fruits.

Flowers treated with 1% naphthoxyacetic acid developed many seedless fruits. In contrast with those resulting from treatment with 4-chlorophenoxyacetic acid, these fruits were excellent in texture and the locules were well filled. Although most of the fruits were entirely seedless, a few had occasional seeds. Early growth of the fruits was greatly stimulated by treatment with this compound. Later experiments showed, however, that a greater set can be obtained when the concentrations are lower than 1%.

Flowers treated with an aerosol of indolebutyric acid at 1% concentration developed seeded fruits, and no increase in fruit-set or in the total number of fruits per cluster was noted. In another experiment with the Marglobe variety, aqueous lanolin-emulsion sprays containing 0.2% indolebutyric acid resulted in a significantly higher fruit-set over control plants.

FIG. 6.—Deformed tomatoes resulting from treatment with 4-chlorophenoxyacetic acid at 1% concentration. Carpel walls have developed, but placentas have developed slowly so that locules are not well filled.

EXPERIMENT III: AEROSOL APPLICATIONS TO SUCCESSIVE CLUSTERS OF FLOWERS

Plants of Rutgers variety were started at the same time as the three varieties used in the preceding experiment, but these plants grew more slowly and as a result the first flowers opened about a week later. Since no data had then been

obtained from the previous experiment, the same growth substances and concentrations were used. Each successive cluster was treated when many of the flowers were open to study the effect of repeated applications of aerosols of growth-regulating substances on individual plants.

Twelve plants were used for each of the five treatments. Application to the first clusters was made February 7, and the plants were then equally divided on two greenhouse benches. The next application, for the second and third clusters, was made February 24. On March 13 the fourth and fifth clusters were treated.

TABLE 4

EFFECT OF AEROSOL SPRAY AT CONCENTRATIONS OF 1% ON FRUIT-SET OF SUCCESSIVE CLUSTERS OF RUTGERS TOMATO. SUCCESSIVE APPLICATIONS ON FEBRUARY 7, FEBRUARY 24, AND MARCH 15, 1944

Treatment	Cluster number	No. of flowers and buds treated*	No. of flowers open*	No. of fruits set	Av. diameter of fruit-set at time of harvest (inches)
4-Chlorophenoxyacetic acid	1	59	30	40	2.46
	2	76	61	40	2.18
	3	47	26	25	2.10
	4	40	29	10	1.91
	5	20	12	6	1.17
Total		242	158	121	2.18†
Naphthoxyacetic acid	1	60	26	14	2.72
	2	69	23	42	2.25
	3	65	39	17	1.81
	4	38	35	6	1.56
	5	19	16	5	1.20
Total		251	139	84	2.13†
Indolebutyric acid	1	63	31	17	2.86
	2	80	72	15	2.24
	3	67	33	12	2.21
	4	60	51	9	2.14
	5	26	16	3	0.83
Total		296	203	56	2.33†
Aerosol control, cyclohexanone+dimethyl ether	1	61	27	10	2.37
	2	55	34	9	2.35
	3	62	42	5	2.30
	4	46	46	10	1.43
	5	23	20	7	1.47
Total		247	169	41	1.97†
Untreated	1	58	28	10	2.67
	2	67	60	14	2.02
	3	61	36	18	2.20
	4	54	44	9	2.00
	5	34	26	4	1.18
Total		274	194	55	2.13†

* Totals for twelve plants. † Average.

For each treatment the plants were removed from the greenhouse to a small ventilated room and sprayed as in experiment II. Not all the plants had clusters

FIG. 7.—Three successive clusters of fruits derived from flowers treated February 7 and February 24 with aerosol of 4-chlorophenoxyacetic acid at 1% concentration.

in the same stage of development. Some had flowers which were classified as overmature; that is, the petals were dry and the style had fallen off. Other flowers either had not yet opened or were in the bud stage.

RESULTS.—With the Rutgers variety (table 4), 4-chlorophenoxyacetic acid, at 1% concentration, was again the most effective compound in stimulating fruit-set, but here also many cavities were found in the mature fruits and increased decay was noted. Naphthoxyacetic acid, as in the previous experiment, was also very effective. Flowers sprayed with indolebutyric acid showed very little response; no increase in set of fruits was noted, and of the fruits that set only a very few were seedless.

The Rutgers plants treated three times with 4-chlorophenoxyacetic acid (fig. 7) set an average of 3.3 fruits per cluster on the first two clusters and an average of 2.1 on the third cluster. The fourth and fifth clusters had an average of less than one fruit per cluster. This may have resulted in part from the fact that the 1% concentration of the acid was so high that much bud inhibition and malformation of the leaves resulted. The fruits of all clusters, although usually normal in appearance, were not well filled, and in some cases decay in the center had developed. Occasionally ovaries of flowers that were classified as overmature were stimulated and partially seedless fruits developed. Seeds often developed in only one locule. The two control lots averaged less than one fruit per cluster.

EXPERIMENT IV: CONCENTRATION OF GROWTH REGULATORS IN AEROSOL SOLUTIONS

It was apparent from results obtained in experiments II and III that the concentration of growth substance in the aerosol solution was too high, and four varieties of tomatoes—Bonny Best, Marglobe, Rutgers, and Pan America—were used to test the effect of concentration. Five growth substances—4-chloro-

phenoxyacetic acid, β-naphthoxyacetic acid, indolebutyric acid, indoleacetic acid, and α-naphthaleneacetic acid—were applied, each at concentrations of 0.05, 0.1, and 0.5%. The plants received these treatments when two or three flowers of the first cluster had opened. Ten plants from each variety were used for each treatment. The aerosol solution was prepared in a pressure test-tube similar to that of figure 1*B*, but of larger capacity. The weighed amounts of growth substances were dissolved in 0.75 gm. of cyclohexanone. This solution was placed in the test-tube and 14.25 gm. of methyl ether forced into the tube as previously described. Approximately 0.5 gm. of the aerosol was used for each cluster. The spraying operation was similar to that shown in figure 5. Before the plants were treated, the total numbers of flowers and buds per plant were recorded, as well as the number of open flowers. Ten days after treatment the fruits were harvested. At this time the number and size as well as the internal condition of the fruits (seeds or seedless) were recorded.

RESULTS.—All three concentrations of 4-chlorophenoxyacetic acid were effective in stimulating the set of fruit (table 5). At concentrations of 0.5 and 0.1%, injury and deformity of the stem and leaves were noted; the 0.05 concentration resulted in only very slight deformation of the leaves. All four varieties responded to this 0.05% treatment, and early growth of the fruits was significantly stimulated. Ten days after treatment, all the young fruits were seedless. The control fruits at this stage had visible (determinable) seeds.

Naphthoxyacetic acid was also very effective, and no injury or formative effects were noted at any of the concentrations tested. When applied at 0.05% it was effective in setting fruit on Pan America, Marglobe, and Rutgers, but it was not very effective in setting seedless fruit on Bonny Best. This concentration seemed to be near the lower limit of effectiveness.

Indolebutyric acid, indoleacetic acid, and α-naphthaleneacetic acid were not consistently effective in increasing fruit-set or in inducing development of seedlessness with any of the four varieties. Naphthaleneacetic acid when applied at 0.5% concentration inhibited fruit-set and enlargement.

EXPERIMENT V: OTHER DILUTIONS OF 4-CHLOROPHENOXYACETIC ACID

The previous experiments using 4-chlorophenoxyacetic acid at 0.5, 0.1, and 0.05% had caused injury and inhibition; hence greater dilutions were necessary. Three concentrations were used—0.01, 0.001, and 0.0001% in a methyl ether–cyclohexanone aerosol. Three varieties, Bonny Best, Pan America, and Rutgers, were employed. The plants were grown during March, with ten plants of each variety in each treatment. The first clusters were treated by direct spray shortly after the flowers began to open. Ten comparable plants of each variety were used as controls.

RESULTS.—No injury was noted to leaves or stems at any of the three concentrations. A significant increase in set over the controls was found with all concentrations (table 6). A greater set, however, was recorded at 0.01% than at either 0.001 or 0.0001%. Some of the fruits that developed in the treatment with 0.0001% contained seeds, indicating that the lower limit of effectiveness was reached. It was also noted that even at the very low dilutions some of the fruits were poorly filled.

TABLE 5

EFFECT OF CONCENTRATIONS OF GROWTH-REGULATING SUBSTANCES AS AEROSOLS ON FRUIT-SET OF FIRST CLUSTER OF TOMATOES. GREEN FRUITS HARVESTED 10 DAYS AFTER TREATMENT

Treatment	Total no. flowers and buds treated*	Flowers open when treated (%)	No. of fruits set*	No. of fruits with seeds*	No. of fruits without seeds*	Av. diameter of fruits 10 days after treatment (inches)
Bonny Best						
4-Chlorophenoxyacetic acid						
0.5%	48	44	29	0	29	.....
0.1%	53	45	28	0	28	.....
0.05%	53	40	12	0	12	0.8
Naphthoxyacetic acid						
0.5%	52	46	25	1	24	0.9
0.1%	45	56	28	3	25	0.8
0.05%	53	54	12	12	0	0.7
Indoleacetic acid						
0.5%	53	45	14	13	1	0.7
0.1%	52	42	16	14	2	0.7
0.05%	47	55	17	15	2	0.6
Naphthaleneacetic acid						
0.5%	45	38	6	0	6	0.7
0.1%	47	43	15	8	7	0.7
0.05%	57	44	17	16	1	0.7
Indolebutyric acid						
0.5%	52	42	14	11	3	0.8
0.1%	50	46	19	19	0	0.6
0.05%	50	44	16	14	2	0.8
Untreated	52	44	10	10	0	0.6
Marglobe						
4-Chlorophenoxyacetic acid						
0.5%	49	46.9	19	0	19	0.4
0.1%	49	48.9	22	0	22	0.8
0.05%	53	41.5	23	0	23	0.9
Naphthoxyacetic acid						
0.5%	52	42.3	18	3	15	0.9
0.1%	48	45.8	17	3	14	0.8
0.05%	48	52.0	21	0	21	0.8
Indoleacetic acid						
0.5%	46	41.3	5	5	0	0.6
0.1%	49	48.9	7	7	0	0.6
0.05%	39	55	8	8	0	0.5
Naphthaleneacetic acid						
0.5%	43	65	2	2	0	0.6
0.1%	46	59	0	0	0	0.5
0.05%	42	60	2	2	0	.....
Indolebutyric acid						
0.5%	45	56	6	6	0	0.7
0.1%	41	60	8	8	0	0.8
0.05%	48	66	16	16	0	0.7
Untreated	52	42	8	8	0	0.6

* Totals for ten plants.

TABLE 5—*Continued*

Treatment	Total no. flowers and buds treated*	Flowers open when treated (%)	No. of fruits set*	No. of fruits with seeds*	No. of fruits without seeds*	Av. diameter of fruits 10 days after treatment (inches)
Rutgers						
4-Chlorophenoxyacetic acid						
0.5%	60	33	22	0	22	0.6
0.1%	57	39	26	0	26	0.6
0.05%	54	46	22	0	22	0.6
Naphthoxyacetic acid						
0.5%	58	38	17	1	16	0.9
0.1%	55	45	21	0	21	0.9
0.05%	56	43	22	0	22	0.8
Indoleacetic acid						
0.5%	38	55	7	7	0	0.8
0.1%	51	35	6	6	0	0.8
0.05%	49	41	6	6	0	0.8
Naphthaleneacetic acid						
0.5%	39	46	0	0	0	0.0
0.1%	31	48	5	5	0	0.6
0.05%	37	56	7	7	0	0.5
Indolebutyric acid						
0.5%	60	32	8	6	2	0.7
0.1%	62	21	10	10	0	0.5
0.05%	55	31	5	5	0	0.7
Untreated	40	43	5	5	0	0.6
Pan America						
4-Chlorophenoxyacetic acid						
0.5%	39	59	18	0	18	.....
0.1%	42	55	23	0	23	.....
0.05%	42	60	21	0	21	.....
Naphthoxyacetic acid						
0.5%	50	38	17	0	17	0.7
0.1%	49	43	18	0	18	0.7
0.05%	54	28	15	0	15	0.8
Indoleacetic acid						
0.5%	51	55	4	4	0	0.5
0.1%	53	43	5	5	0	0.6
0.05%	45	56	4	4	0	0.6
Naphthaleneacetic acid						
0.5%	54	52	0	0	0	.....
0.1%	50	58	0	0	0	0.5
0.05%	53	47	9	9	0	0.7
Indolebutyric acid						
0.5%	53	32	2	2	0	0.7
0.1%	50	28	6	6	0	0.6
0.05%	52	35	9	8	1	0.6
Untreated	50	42	5	5	0	0.5

Experiment VI: Aerosol fumigation with 4-chlorophenoxyacetic acid

This experiment was designed to test the effect of an aerosol-vapor treatment, using two dosages of chlorophenoxyacetic acid. Three varieties were used—Bonny Best, Marglobe, and Pan America. The plants were grown during March and April until the first clusters each had two or three flowers open. Ten plants from each variety were used for each experiment. Two small rooms, capacity 1000 cu. ft., were employed, and into each room a group of thirty plants was taken. In one room an aerosol was released containing 25 mg. of chlorophenoxyacetic acid, in the other room one containing 50 mg. The same amount of methyl ether and cyclohexanone was released in each room. The plants were kept in the rooms for 16 hours and were then returned to the greenhouse benches and allowed to grow. The young fruits were harvested 10 days later, and records of size and condition of seedlessness were recorded.

Results.—Seedlessness and greatly increased fruit-set were obtained by using 4-chlorophenoxyacetic acid as a aerosol-vapor treatment (table 7). No injury to the foliage was noted at either concentration. All three varieties responded to the treatment. Some of the plants exposed to the higher concentration of 50 mg. per 1000 cu. ft. showed slight epinasty. At greater concentrations the normal vegetative habit of the plant would probably be impaired. For the three varieties only thirteen fruits were set on a total of thirty untreated plants, while with the treatment at 50 mg. of chlorophenoxyacetic acid per 1000 cu. ft. of space, sixty-five fruits were set.

TABLE 6

Effect of various concentrations of 4-chlorophenoxyacetic acid as aerosol on set and development of fruits of tomatoes. Fruit harvested 10 days after treatment

Treatment	Total no. flowers and buds treated*	Total no. flowers open when treated*	Total fruit-set at end of 10 days*	Av. diameter of fruits 10 days after treatment (inches)
Bonny Best				
0.01%	46	13	23	0.5
0.001%	43	11	12	0.4
0.0001%	51	19	21	0.7
Untreated	52	17	6	0.4
Pan America				
0.01%	47	27	13	0.5
0.001%	50	28	15	0.7
0.0001%	55	32	11	0.5
Untreated	46	28	4	0.6
Rutgers				
0.01%	63	29	29	.....
0.001%	62	31	22	0.9
0.0001%	59	26	23	0.9
Untreated	56	30	17	0.8

* Totals for ten plants.

Discussion

Increased set of tomato fruits and in many cases production of seedless fruits can be obtained by application to the flowers of certain growth-regulating substances dispersed as aerosols. These compounds vary in their effectiveness. 4-Chlorophenoxyacetic acid was extremely effective, a concentration as low as 0.001% being sufficient to set fruit. The locules of the fruits resulting from treatment with this substance, however, are usually poorly filled. Naphthoxyacetic acid is also very effective, but the necessary concentration appears higher than that of chlorophenoxyacetic acid. Indolebutyric acid and naphthaleneacetic acid have not been consistently effective, either in promoting fruit-set or in stimulating development.

The advantage of the aerosol method appears to arise from its rapidity and simplicity of application, once the materials have been prepared in suitable containers. A plant or tree can be treated in a few seconds merely by opening a valve. Very small amounts can be used for effective treatment and a uniform application made without injury or distortion of the plant if the correct concentrations are used.

Cyclohexanone itself applied in any large amount is toxic to tomato plants, but the small amounts deposited on the plant by the aerosol treatment have shown no harmful effects.

TABLE 7

EFFECT OF 4-CHLOROPHENOXYACETIC ACID APPLIED AS FUMIGATION ON SET AND DEVELOPMENT OF FRUITS OF TOMATOES. FRUIT HARVEST 10 DAYS AFTER TREATMENT

Treatment	Total no. flowers and buds treated*	Total no. flowers open when treated*	Total no. fruits set after 10 days*	Av. diameter of fruits 10 days after treatment (inches)
Bonny Best				
25 mg./1000 cu. ft.	53	23	19	0.7
50 mg./1000 cu. ft.	47	26	26	0.7
Control	54	15	7	0.6
Pan America				
25 mg./1000 cu. ft.	54	24	22	0.6
50 mg./1000 cu. ft.	47	28	26	0.6
Control	53	26	4	0.5
Marglobe				
25 mg./1000 cu. ft.	39	26	12	0.7
50 mg./1000 cu. ft.	45	26	13	0.6
Control	59	30	2	0.6

* Totals for ten plants.

HOWLETT (5) has reported increased set of fruit by the use of indolebutyric acid in an aqueous lanolin emulsion. We have repeated some of his experiments and have also noted an increased set of fruit. In an aerosol, however, indolebutyric acid appeared very ineffective in increasing fruit-set. The reason for this is not apparent.

Although these experiments have dealt largely with methyl ether as the vapor carrier, it is possible to use Freon,

either in a mixture of Freon and methyl ether together with the common growth substances, or of Freon with some of the esters of the growth substances. Methyl ether has been used largely because most of the growth substances are readily soluble in it. It has certain disadvantages, but for this purpose they are not great. At very high concentrations (50% or higher) in the atmosphere it is toxic to human beings. At 3% in the atmosphere it is inflammable. All of our work involved concentrations much lower than 3%.

The seedless tomato fruits developed by the use of aerosol method were of excellent quality. In experiments recently completed but not reported here, in which both 4-chlorophenoxyacetic acid at 0.01% and β-naphthoxyacetic acid at 0.25% were used together in aerosol, a very good set was obtained, and all the fruits were of excellent quality and well filled.

Summary

1. A recently developed aerosol method of applying growth-regulating substances to tomatoes has proved an effective means of inducing fruit setting and, in many cases, of producing seedless fruits.

2. 4-Chlorophenoxyacetic, β-naphthoxyacetic, indoleacetic, indolebutyric, and α-napthaleneacetic acids differed in their capacities to induce fruit-set. Of these, 4-chlorophenoxyacetic acid was the most effective in setting fruit and stimulating early fruit growth. When used at concentrations higher than 0.01%, the treated plants developed abnormal leaves and fruits.

3. β-naphthoxyacetic acid also induced setting of seedless tomatoes when applied as an aerosol. The safest and still effective range of concentration lies between 0.01 and 0.5%. Not all the fruits from treated flowers were seedless. Apparently their age and the amount of pollination determined to some extent the amount of seed development, but early fruit growth was greatly stimulated.

4. Indoleacetic, indolebutyric, and α-naphthaleneacetic acids were less effective than the other compounds in inducing fruit-set and producing seedless fruit when applied by this aerosol method.

Bureau of Plant Industry, Soils and Agricultural Engineering
Beltsville, Maryland

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EFFECTS OF FLUORESCEIN AND PHOTOSENSIN ON GROWTH OF RED KIDNEY BEANS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 563

ROBERT K. ZUCK

Introduction

Many organic compounds are known to have local growth-regulating effects on plants. Attempts to disclose general stimulation of growth by these compounds have been undertaken many times, but critical work has revealed little or no stimulation of the growth of the entire plant by any of the compounds examined (**2, 8**). Among those tested were indoleacetic acid, indolebutyric acid, naphthaleneacetic acid, naphthalene acetamide, and phenylacetic acid. At the present time there is apparently no experimental evidence indicating stimulation of growth of the entire plant by this class of growth-regulating substances.

Recently it has been stated (**6, 7**) that fluorescein acts as an inhibitor and as a stimulator of plant growth, depending upon its concentration. This compound is not known to have local growth-regulating effects. In their work with fluorescein, SELLEI, SELLEI, MAYER, and WENT (**7**) have employed a commercial preparation (Photosensin) composed of 93.9% sodium bicarbonate, 5% fluorescein, 1% iron sulphate, and 0.1% copper sulphate. Where inhibition or stimulation of plant growth has resulted from the application of varying amounts of Photosensin, the effects on the plant have been attributed to the fluorescein content and not to any one or all of the other three compounds of the preparation. In the one case where Photosensin was not used, fluorescein was presumably employed with sodium bicarbonate to keep the high concentrations of fluorescein in solution. In addition, the experiments were carried out on a small number of plants for each treatment where a synthetic substratum was used. Five plants per treatment are not sufficient to overcome individual variation in the case of such variable species as marigold and tomato when grown from seed.

The experiments reported here were designed to investigate further the effects of fluorescein, of Photosensin, and of the inorganic constituents of Photosensin on more extensive populations of a single variety of plant grown on a synthetic substratum.

Material and methods

Red kidney beans were grown under greenhouse conditions at two separate times, from October 9 to November 6, 1942; and from May 25 to June 14, 1943. In both cases growth was terminated when flower buds began to appear. The lot of seeds employed in the 1942 experiment proved irregular in germination, emergence, and development. The epicotyls of many seedlings did not elongate or continue development, although some plants produced shoots from buds in the axils of the cotyledons. Only those plants in which the epicotyl continued to develop were used, however, those with shoots produced from buds in the axils of cotyledons being discarded. A second lot of seed employed for the experiment of 1943 gave a very uniform stand of vigorous plants, of which the first simple leaves were exceptionally large.

Two plants were grown in 5 lb. each of pure quartz sand in glazed jars with side drainage at the bottom, the hole being covered with glass wool. Seeds

were planted four to a jar at 1-inch depth and immediately supplied with complete nutrient, the sand previously having been rinsed twice with distilled water. Just after the hypocotyl arch had straightened out, the seedlings were thinned to two per jar.

The nutrient medium consisted of 0.0060 M calcium nitrate, 0.0045 M magnesium sulphate, and 0.0045 M potassium-acid phosphate in distilled water. Microelements were supplied as 0.1 p.p.m. iron sulphate, 1.0 p.p.m. zinc chloride, 1.0 p.p.m. manganese sulphate, and 1.0 p.p.m. sodium tetraborate. No copper was added to the first series but was added as 1:50,000,000 copper sulphate in the second experiment to keep the medium comparable with that used by SELLEI *et al.* (7). All chemicals were of c.p. grade. The fluorescein was obtained from Eastman Company and the Photosensin was the commercial product of that name.

Hydrogen and hydroxylion determinations were made on the Coleman glass electrode electrometer no. 3. Osmotic pressures were determined according to the freezing-point depression method of LOOMIS and SHULL (4).

Harvesting was by rows of seven jars each, the tops being cut off at sand level and the roots rinsed free of sand in tap water. Both tops and roots were dried in a hot-air oven at 60°–70° C. for 18 hours. The dried samples were then placed in a desiccator over calcium chloride until constant weights were obtained. Weights were taken to the nearest hundredth of a gram.

The first experiment consisted of two treatments and a control. The concentrations of fluorescein were 1:50,000 and 1:500,000, the control being plain nutrient. There was some precipitation of the fluorescein at the 1:50,000 concentration, but this was stirred up each time the solution was applied. The pH of the nutrient was 4.0 and was not affected by the addition of the fluorescein. Each treatment comprised 140 plants arranged in ten rows of seven jars each, the resulting thirty rows randomized on one bench in the greenhouse. Randomization was accomplished by assigning numbers to the treatments, shaking them in a container, and drawing them off until each row had been assigned the type of treatment. The plants were thinned to two per jar and treatment begun on October 15, 1942, 6 days after the seeds were placed in the sand. Solutions of the corresponding type were applied when the surface of the sand began to appear dry. This amounted to five applications of about 250 ml. each. The plants were harvested on November 6, 1942.

The five treatments (and a control) of the second experiment, the plants randomized as in the first experiment, were designed to observe the effect of a concentration of fluorescein of 1:10,000,000 and the accompanying amounts of inorganic compounds found in the quantity of Photosensin required to obtain this concentration of fluorescein. Each treatment and control consisted of 140 plants, following the method of the first experiment. A concentration of fluorescein of 1:10,000,000 was chosen, since the data of SELLEI (7) indicate this to be near the optimum. The treatment consisted of the following concentrations in nutrient solution: fluorescein 1:10,000,000; sodium bicarbonate 18.8:10,000,000; iron sulphate 0.2:10,000,000; copper sulphate 0.02:10,000,000; Photosensin to make 1:10,000,000 fluorescein.

The pH of the five dilute solutions of the second experiment was taken before and after addition of the substances. No

changes were observed. Four of the solutions were pH 4.0, the one with sodium bicarbonate being pH 3.95. Thinning of the plants and application of the treatments was done in May, 1943. The longer growing period required seven applications of about 250 ml. Harvesting occupied 3 days. All tops were cut on June 14, 1943. The roots were harvested on the two succeeding days, one bench on each day.

A third experiment was run on a smaller scale to observe the effects of considerable amounts of sodium bicarbonate over a range of concentrations with and without fluorescein, as this compound is the largest (93.9%) constituent of Photosensin. The sodium bicarbonate and fluorescein concentrations per liter of nutrient, together with the pH, were as follows:

Sodium bicarbonate		
56.4 gm.,	without	fluorescein, pH 8.40
56.4	with 3 gm.	pH 7.90
18.8	without	pH 8.40
18.8	with 1 gm.	pH 7.95
9.4	without	pH 8.30
9.4	with 0.5 gm.	pH 8.20
4.7	without	pH 8.05
4.7	with 0.25 gm.	pH 8.75

The solutions were applied to two jars of two to four plants for each treatment. The osmotic pressure of the solution containing 56.4 gm. of sodium bicarbonate per liter of nutrient was 24.85 atmospheres; that of the same amount of sodium bicarbonate plus 3 gm. of fluorescein was 21.39 atmospheres. No weights were taken on the plants of this experiment, since they were killed by the three highest concentrations of sodium bicarbonate and of it plus fluorescein. One application of each treatment was sufficient to produce the effects noted.

Results

In the first experiment employing only fluorescein, no consistent differences were noticeable among the living plants. Some irregularities occurred, but these could not be related to any treatment. Table 1 lists the dry weights of roots and tops, each weight representing the tops or roots of fourteen plants, together with the total weights of tops and roots for each treatment. The weight of the plants treated with the 1:500,000 dilution is 12.14 gm. less than the control, and the plants treated with the 1:50,000 dilution weigh 6.58 gm. more than the control. On a percentage basis, the plants of 1:50,000 dilution weigh 3.8% greater, and those treated with the 1:500,000 dilution weigh 6.2% less, than the controls.

TABLE 1

FLUORESCEIN IN RELATION TO GROWTH OF RED KIDNEY BEANS. DATA IN GRAMS DRY WEIGHT OF FOURTEEN PLANTS

CONTROL (PLAIN NUTRIENT)		FLUORESCEIN IN NUTRIENT SOLUTION			
		1:50,000		1:500,000	
Tops	Roots	Tops	Roots	Tops	Roots
12.54	3.90	12.66	1.04	13.54	2.30
14.60	3.12	14.68	1.97	18.87	2.84
13.56	3.04	12.20	1.55	12.31	3.14
14.13	3.51	15.48	3.80	9.31	2.83
11.91	2.95	16.16	5.94	14.25	5.94
11.22	3.67	14.91	3.84	12.67	5.02
12.36	2.50	11.89	5.43	12.53	4.72
13.91	3.57	13.39	5.35	10.57	5.70
13.06	4.57	14.58	4.60	9.01	2.52
16.94	4.94	11.94	4.97	12.15	4.44
134.23	35.57	137.89	38.49	118.21	39.45
Total..	169.80	Total..	176.38	Total..	157.66

In comparing the data in tables 1 and 2, greater regularity in weights are shown in table 2, indicating an even

more uniform stand of plants. Likewise, no visible differences were observed in the growing plants of the second experiment. The copper-sulphate treated plants of the second experiment weighed the most, with the fluorescein- and Photosensin-treated plants second and third highest in weight, respectively. The plants of the control and those treated with iron sulphate and sodium bicarbonate were all very nearly the same weight.

The sodium-bicarbonate treatments of the third experiment with and without fluorescein in the three highest concentrations killed the plants. The highest concentration of sodium bicarbonate alone, and with the 3 gm. of fluorescein, produced noticeable effects on the plants within 5 minutes after application. At the end of 15 minutes the plants had collapsed. Fluorescein began to show in the veins within half an hour after treatment, indicating that the root systems were killed. All plants receiving fluorescein plus sodium bicarbonate and sodium bicarbonate alone in the three highest concentrations were dead 1 day after treatment. Those receiving the lowest concentrations showed drying at the edges of the oldest leaves at the end of 2 days, when the experiment was terminated. The plants were of the same stage of development as were those of the first and second experiments when treatments began; that is, the hypocotyl arch had just straightened out.

TABLE 2

GROWTH OF RED KIDNEY BEANS IN RELATION TO FLUORESCEIN, PHOTOSENSIN, AND INORGANIC CONSTITUENTS OF PHOTOSENSIN. CONCENTRATIONS AS INDICATED IN NUTRIENT SOLUTION. DATA IN GRAMS DRY WEIGHT OF FOURTEEN PLANTS

CONTROL (PLAIN NUTRIENT)		0.2:10,000,000 IRON SULPHATE		1:10,000,000 FLUORESCEIN		18.8:10,000,000 SODIUM BICARBONATE		0.02:10,000,000 COPPER SULPHATE		PHOTOSENSIN TO MAKE 1:10,000,000 PHOTOSENSIN	
Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots
20.61	5.13	21.28	4.17	22.07	4.60	21.93	4.74	23.87	5.35	20.47	4.95
23.31	7.07	23.20	6.19	22.29	5.20	22.59	4.65	23.30	4.51	23.73	6.03
22.96	5.28	23.38	5.63	23.23	4.71	20.27	6.78	23.65	5.14	23.14	4.68
18.47	4.95	21.56	4.45	21.73	4.64	21.33	4.44	23.43	5.88	23.75	5.38
24.29	5.15	23.26	4.85	21.93	4.35	20.90	5.02	24.10	5.14	21.82	4.12
20.75	5.19	22.04	4.56	22.60	4.40	22.54	4.54	22.71	4.81	22.14	4.16
20.95	4.56	21.99	4.63	25.19	5.95	23.77	4.75	22.48	4.29	21.54	4.79
22.98	4.95	22.49	4.26	23.33	4.55	21.50	4.04	23.73	5.65	22.30	4.93
21.51	4.46	21.81	4.81	22.51	4.47	22.74	4.64	21.12	4.19	21.97	5.00
21.21	5.17	20.95	4.82	22.20	5.28	22.55	5.00	22.18	5.17	23.15	5.72
217.04	51.91	221.96	48.37	227.08	48.15	220.12	48.60	230.57	50.13	224.01	49.76
Total..	268.95	Total..	270.33	Total..	275.23	Total..	268.72	Total..	280.70	Total..	273.77

Discussion

In considering the data presented by SELLEI *et al.* (7) for the sand culture experiments consisting of five plants per treatment, a lack of consistency over the range of treatments is apparent. Although most of the treated plants developed higher weights than the controls, these in several instances are higher than the treated plants. Furthermore,

the series of treatments do not form a smooth curve of increasing weights with decreasing concentrations, or vice versa. Nor can the effect on the plants logically be attributed to the fluorescein in the Photosensin preparation employed by them. As was pointed out earlier in this paper, Photosensin is composed of four compounds, three inorganic ones and the organic fluorescein. The inorganic compounds have cations definitely known to have effects on the growth of plants, the anions not being important in the concentrations used. Likewise, the stunting effect attributed to the fluorescein in high concentrations cannot be divorced from the inorganic compounds of the Photosensin preparation.

The lack of pronounced visible differences among the living plants and the small differences in dry weights shown in tables 1 and 2 indicate that fluorescein by itself in the three concentrations employed is without appreciable effect on the growth of red kidney beans. If the slight additional weight made by the plants treated with the 1:50,000 fluorescein concentration was attributable to the dye, then the slightly less weight made by the plants receiving 1:500,000 and 1:10,000,000 fluorescein cannot easily be explained as an actual inhibition of growth. Rather, the small differences in dry weights and appearance of the living plants indicate that these are within the variations of the populations of plants employed.

The data supplied by the second experiment do not appear to show significant differences, inasmuch as the greatest weight was made by those plants receiving the slight additional copper found at a concentration of fluorescein of 1:10,000,000 as Photosensin. In all probability, the variations in weights are those to be expected with populations of this size under ordinary conditions.

Sodium, which is in dispute as an essential element for plants, is not without effect on plants and cannot be ignored when used in low concentrations as sodium bicarbonate in the Photosensin preparation. MULLISON and MULLISON (5), working with barley, found evidence of substitution for potassium at almost all levels of potassium and sodium.

Any further experimentation to observe the effects of fluorescein as a stimulator of plant growth must take into account that Photosensin is a preparation consisting of fluorescein, sodium bicarbonate, iron sulphate, and copper sulphate. If the effects are to be referred to fluorescein, this dye must be employed by itself.

Those plants receiving the high concentration of sodium bicarbonate and sodium bicarbonate-fluorescein in nutrient solution were killed by the treatments. The effects on the plants cannot be called merely stunting of growth, for death ensued within a matter of minutes after the plants had been subjected to the treatments. The killing is about as rapid when the sodium bicarbonate-fluorescein solutions are used as when the sodium bicarbonate alone is applied. Although the pH values are relatively alkaline for good growth, the rapidity with which these plants were killed points rather to the high osmotic pressures (24.85 atmospheres for the highest concentration of sodium bicarbonate) to which the roots were subjected. The precipitation of much of the magnesium and calcium in the form of carbonates by the extensive amounts of sodium bicarbonate removes the buffering qualities of these metallic ions from the solu-

tion. Likewise, the nitrate and sulphate ions released by these precipitated cations allow for more thorough ionization of the sodium in solution. All these factors probably contribute to the rapidity with which the plants are killed, but it would appear that the high osmotic pressures are largely responsible.

HAYWARD and LONG (3) and GERNER (1), working with high osmotic pressures attained by sodium sulphate and sodium chloride in nutrient solution, found that killing was common in the higher concentrations of these salts. HAYWARD and LONG report that killing was complete for tomato at an osmotic pressure of 6 atmospheres attained by the addition of sodium sulphate to the nutrient solution, whereas GERNER reports killing as very frequent for tomato at the highest concentrations, between 11 and 12 atmospheres. In both papers it is stated that the high osmotic values were attained by gradually increasing the amounts of solutes over a period of days. HAYWARD and LONG do not report a sodium toxicity as such, but they do note that "it seems possible, however, that the sodium ion may have an inhibitory effect on growth."

Thus, it can be assumed that the rapid change in the physico-chemical environment of the root systems of the plants subjected to the extensive amounts of sodium bicarbonate caused death of the plants, and the observed effects cannot be attributed to the fluorescein when it is kept in solution by sodium bicarbonate.

Summary

1. Red kidney beans were grown in pure quartz sand with nutrient solution to which were added in separate series Photosensin and the constituents of Photosensin—fluorescein, iron sulphate, sodium bicarbonate, and copper sulphate.

2. There were no pronounced visible differences among the living plants, and the differences in dry weight were small. Those plants receiving the small amount of copper sulphate weighed the most.

3. Apparently fluorescein does not stimulate growth of red kidney beans.

4. The so-called stunting effect heretofore ascribed to the high concentrations of fluorescein is shown to be actual killing by the extensive amounts of sodium bicarbonate used to keep the fluorescein in solution.

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BELTSVILLE, MARYLAND

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CURRENT LITERATURE

John Merle Coulter: Missionary in Science. By ANDREW DENNY RODGERS III. Princeton, New Jersey: Princeton University Press, 1944. Pp. 321.

This is not a biography in the usual sense of the term, but rather a constructive and illuminating contribution to the history of botany in the United States through the life-span of one individual (1851-1928). It traces his influence, in collaboration with his contemporaries, upon the course and development of botanical science—particularly systematics and morphology—during that period. The discussion and territory covered range far, and as first one individual and then another is discussed, the central figure—Professor JOHN MERLE COULTER—often becomes obscured, although the theme is maintained. The names of ARTHUR, BAILEY, BESSEY, BRITTON, COVILLE, COWLES, FARLOW, GRAY, MACDOUGAL, ROSE, and a score of others cross the pages with such frequency, and are often treated at such great length, that touch with the personality of Dr. COULTER is all but lost until near the end of the book. But despite these complications, the attentive reader readily weaves it all into a clear, vivid history of an unusually active, formative period of botanical development.

The characterization of Dr. COULTER which eventually emerges differs in many details from that seemingly carried by a large body of his students. The author paints him, not simply as a great teacher, editor, and scientist aloof from application and practicality, but—through quotations and by other means—as emphasizing early in his career and again and again later the practical values and economies to be derived from the application of the principles of pure science. His broad interests in the development of the fields of physiology, ecology, and later those of pathology and genetics are stressed. Dr. COULTER's contributions through lectures and writings to the field of secondary and advanced education are detailed at length. The reasons and events which led to the broadening of his earlier interests in systematics to include morphology and other phases of botanical endeavor are pointed out. Emphasis is placed on the fact that Dr. COULTER's deeply religious interests never wavered, and on the fact that he found no disharmony between evolutionary and Christian doctrines. Dr. COULTER stressed the relation and application of science to agriculture and to every-day life. Some of his statements made nearly half a century ago are as pertinent, clear, and direct as many which today pass as being strictly modern, daring, and revolutionary!

Despite its trying style, the work has been prepared with great attention to accurate detail. Material from letters, lectures, articles, and other contributions has been extensively drawn upon, and members of Dr. COULTER's family and many of those who had worked with him, either as colleagues or students, were interviewed directly and at length.

As a report on an era of development of botanical science in the United States, the book should occupy a place on the "must" list of botanical reading and study of every individual who desires a clear picture of this science.—E. J. KRAUS.

Mitosis: The Movement of Chromosomes in Cell Division. By FRANZ SCHROEDER. New York: Columbia University Press, 1944. Pp. x+110. Illustrated, $2.00.

This treatise is concerned with a subject to which the author has devoted many years of intensive and fruitful research. It presents a résumé and a critical review of the various hypotheses advanced to account for the mitotic process. In the main, only investigations of the last 20 years are considered in detail, the older being included only when necessary to provide a background. The author discusses the structure of living and fixed cells, the actuality of structural elements, the nature and origin of the spindle apparatus and the hypotheses of mitosis (including contraction, expansion, and their variations), viscosity and hydration, electrostatics, diffusion, streaming, hydrodynamics, tactoids and chromosome autonomy. These topics are presented fairly and without prejudice, with the evidence for and against each clearly brought out. The need for further critical research in this field is forcefully indicated.—J. M. BEAL.

Bibliography of References to the Literature on the Minor Elements and Their Relation to Plant and Animal Nutrition. Fifth Supplement to Third Edition. New York: Chilean Nitrate Educational Bureau, Inc., 1944. Pp. 96.

The fifth supplement contains approximately 700 abstracts, dealing with 117 plants or plant groups and with 48 elements, excluding those treated in the few abstracts of the rare earth and miscellaneous sections at the end. The abstracts are arranged in alphabetical order of the elements. The three indices of the previous supplements—element, botanical, and author—are continued, and a fourth—animal nutrition—is added. These indices add much to the value of the book.—S. V. EATON.